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EFFECT AND SAFETY OF PROBIOTICS TO REDUCE THE INCIDENCE OF ATOPIC DERMATITIS

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Keywords: *Atopic Dermatitis; Staphylococcus aureus; Probiotics; Bifidobacterium; Lactobacillus*

Abstract

This systematic review aims to determine the effect and safety of probiotics in reducing the incidence of Atopic Dermatitis, from the pre/ post-natal period in infants and children or adults, with the most studied and effective probiotics that belong to the genera *Bifidobacterium* and *Lactobacillus.* This study was carried out, through the collection of several scientific articles between the years 2014 to 2019, with the duration of research between September 2019 to June 2020.

Atopic dermatitis is a chronic or recurrent inflammatory skin disease, characterized by dry skin, intense itching, recurrent eczematous lesions and loss of sleep.

The use of probiotics is considered a new therapeutic strategy for the treatment of this atopy. Probiotics are "live microorganisms that confer health benefits to the host when administered in adequate amounts". This produces beneficial effects, when administered in adequate quantities, having a role in the gastrointestinal tract and in the intestine-brain-skin axis and, activating several immunological mechanisms, once that the response is exacerbated in the atopic individual. Therefore, the results demonstrate efficacy, efficiency and safety in reducing the incidence of atopy. However, heterogeneity and other factors influence the atopic dermatitis score values and further studies are needed.

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Introduction

1. Atopic Dermatitis

Atopic dermatitis (AD) is a chronic or recurrent inflammatory skin disease, characterized by dry skin, intense itching, recurrent eczematous lesions and loss of sleep, which affects the patients' quality of life (1–5). However, AD is also known as eczema, according to the World Allergy Organization, to refer to the clinical phenotype of the pathology (6).

This is related to other hypersensitivity reactions, such as high risks of allergy, especially food, asthma, rhinitis and mental health problems (2,7). It is also associated with other pathologies, such as cardiovascular disease and obesity (8).

In the last decades, AD has increased rapidly in the world, with a prevalence of 1-3% in adults and 10-20% in children, with 60% of the cases that start during the first year of life, that is, it represents a great challenge in Pediatric Medicine (9,10). In addition, other studies show that its prevalence has tripled in industrialized countries, affecting 2 to 10% of adults and 15 to 30% of children worldwide, with 20% being infants, in which 45% of cases the disease starts in the first six months of life, 60% during the first year and 85% starts before the age of five (11). Furthermore, it is verified that AD is higher in urban areas than in rural areas (12). However, another theory that justifies the prevalence of AD is the "hygiene hypothesis", which implants the colonization pattern and the diversity of the intestinal microbiota (13,14). Thus, this theory states that in the modern hygienic living conditions, there is a reduction in exposure to microorganisms early in life, which results in inadequate immunological priming (15). Thus, the exposure to bacteria and viruses in the children's environment is a crucial factor in the development of allergy (7,16).

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In addition, in infants and children, the severe form of the disease predominates with the presence of erythema, intense itching and blisters, affecting mainly the face, the extensor surface of the extremities and the scalp (3,4).

Thus, AD is characterized by the presence of skin lesions, excoriation, lichenification, papules, exudation and crusts, that is, morphologically this pathology varies according to age. This way, it can vary according to the darker skin type and also to follicular accentuation, hyper or hypo pigmentation. And finally, itching, which is a very frequent feature of AD, as it leads to the appearance of abrasions causing an increase in inflammation (8).

1.1. Etiology and Anatomophysiological Changes of AD

At the pathophysiological level, AD is a multifactorial disease with an interrelation between the skin barrier, genetic predisposition, immune development and the microbiota composition of the skin. In addition, it also involves environmental, nutritional, pharmacological and psychological factors (Figure 1.), which contribute to the worsening or development of AD (1,17,18).

In AD there are two possible causes that explain its existence, such as: the extrinsic or mediated by Immunoglobulin E (IgE), with high levels of IgE, and the intrinsic or non-IgE mediated, with normal serum levels of IgE (8). The extrinsic hypothesis consists of weakening the skin barrier and in an inadequate differentiation of keratinocytes, which allows the penetration of antigens (Ag). Thus, the Ag causes immunological sensitization and consecutively immune activation. In the intrinsic hypothesis, a weakening of the skin barrier occurs, which promotes the introduction of allergens. Thus, there is an increase in the permeability of the skin barrier and the consequent penetration of allergens and microorganisms (19).

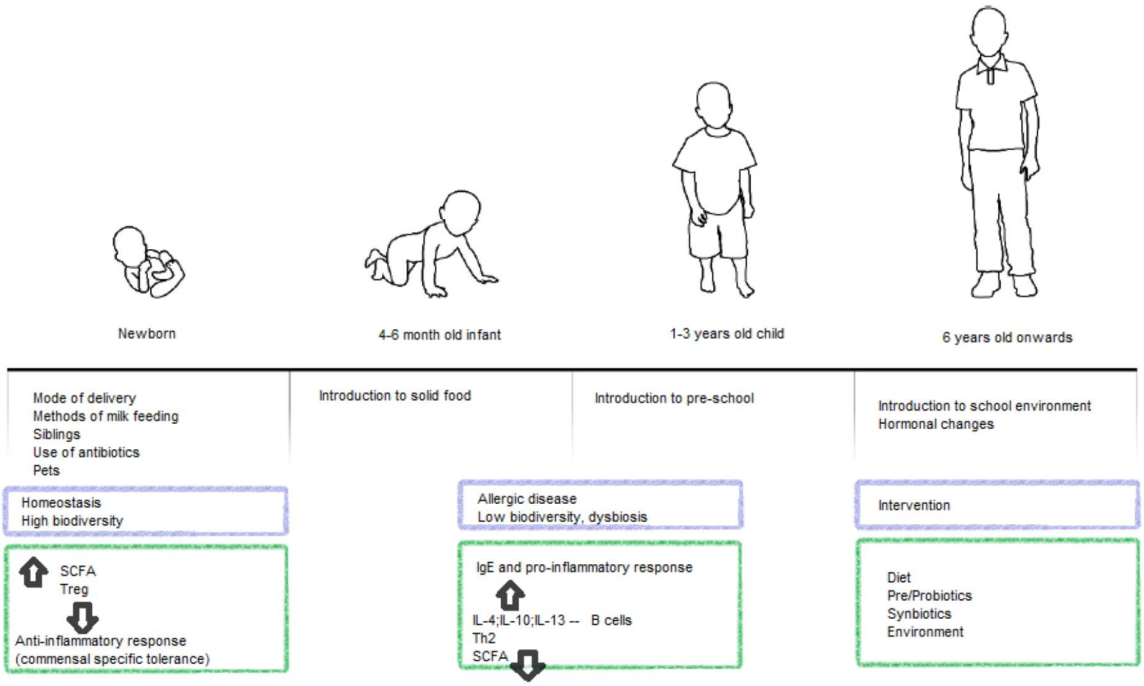

Figure 1. Interaction between the environment and the host. The microbial diversity is associated with several factors, such as vaginal delivery, breastfeeding, interaction with siblings and pets. Therefore, there is an increase in regulatory T cells (Treg cell), short-chain fatty acids (SCFAs) and immune tolerance. In addition, at the beginning of life, there is a low biodiversity that affects the host's immune system, causing a pro-inflammatory response. Source: (Kim 2019)

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However, AD is classified as a biphasic disease, presenting two acute and chronic phases, mainly caused by the responses of T helper 1 (Th1) and T helper 2 (Th2) cells. However, AD is more heterogeneous in the acute phase, which consists on the responses Th2 (interleukin (IL) 4, IL5, IL13, IL31 and CCL-18) and Th22 (proteins IL-22 and S-100A). In contrast, the chronic phase comprises the accentuated acute phase pathways, together with Th1 cells (interferon [IFN] -ʎ, CXCL-9 and CXCL-10) (8).

There are other causes that justify the existence of AD, such as the inflammation of Th2 cells and the unbalance of the epidermal barrier. Thus, it occurs a decrease in filaggrin (FLG) and claudin 1, providing an unbalance in the barrier.

Therefore, FLG encodes a protein that is responsible for retaining moisture and protecting the skin, from environmental allergens (7). This way, FLG is an essential component for the balance of the skin barrier, as its deficiency is associated with an increase in pH, which makes the colonization of *Staphylococcus* aureus (*S*. aureus) favorable (15,20). In addition, there is still some controversy about AD, based on two possible hypotheses: "Inside-Out", due to the dysfunction and systemic inflammation of the epidermal barrier. And finally, "Outside-In", with an epidermal rupture of the skin barrier, activating an immune imbalance (20,21).

In the "Inside-out" hypothesis, there is a skin inflammation, as there is a weakening of the barrier. This happens because there was a decrease in the production of filaggrin. The rupture of the barrier is due to the transcutaneous penetration of allergens, with an increase in *Staphylococcus* aureus (20). In the "Outside-In" hypothesis, there is an immunological dysregulation, because it occurs a filaggrin gene mutation. These mutations in the filaggrin gene occur due to environmental factors, such as temperature and humidity, jeopardizing its production. The rupture of the skin barrier results from an increase in cutaneous and systemic responses of Th2 cells and IL-4 and IL-a3, with thymic stromal lymphopoietin (TSLP). Thus, they are responsible for generating allergic diseases, such as asthma and for the progression of AD to other forms of atopy, such as food allergy (17,20).

1.2. Skin Barrier and Immune System Dysfunction

The first contact of mucosal tissues with the external microbiota is essential for the establishment and maturation of mucosal and systemic immune systems. The evolution of the skin barrier and the immune system are influenced by environmental factors, such as feeding patterns, use of antibiotics by the mother during labor or postnatal use of antibiotics by the newborn (17). In addition, the microbial composition of the skin is affected by several factors, such as: age, sex, exposure to microbial antigens and the type of delivery. Other factors that influence are: pH, temperature, exposure to ultraviolet rays and natural hydration factors, which change easily throughout life (17).

AD demonstrates a suppression of structural proteins and lipids in the stratum of the epidermis, which are essential for barrier function and water retention. The insoluble stratum corneum consists of proteins, including keratin composed of disulfide and gamma glutamyl-lysine linked to structural lattices, such as loricrin, involucrin and proline. Finally, the stratum corneum is also composed of a layer of lipids, such as cholesterol and free fatty acids (21). The protective role of the skin barrier is attributable to antimicrobial peptides (PAMPs), which are small peptides available in high amounts in the skin. There are PAMPs in smaller amounts on the skin of AD (Figure 2.), such as β-defensins, cathelicidin and dermcidin. This is due to the presence of Th2, which causes an increase in colonization by S. aureus. In contrast, coagulase-negative staphylococci (CoNS) that express PAMPs are found in greater amounts in normal skin, as they are rarely detected in AD lesions (15).

FLG helps to maintain epidermal balance and barrier function, by adding keratin filaments, preventing the loss of water and foreign substances. However, its amino acid decomposition products, pyrrolidone carboxylic acid and urocanic acid, promote skin hydration and ultraviolet protection (21). In addition, these acids are responsible for modulating the immune function by decreasing the skin's pH, preventing the activation of serine proteases and the growth of bacteria (21).

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In addition, the decrease in FLG degradation products is caused by the transepidermal water loss (TEWL) and the colonization of allergens, more specifically with S. aureus. Thus, the imbalance of the skin barrier increases the loss of TEWL and the climatic changes in temperature and humidity affect patients with AD (21).

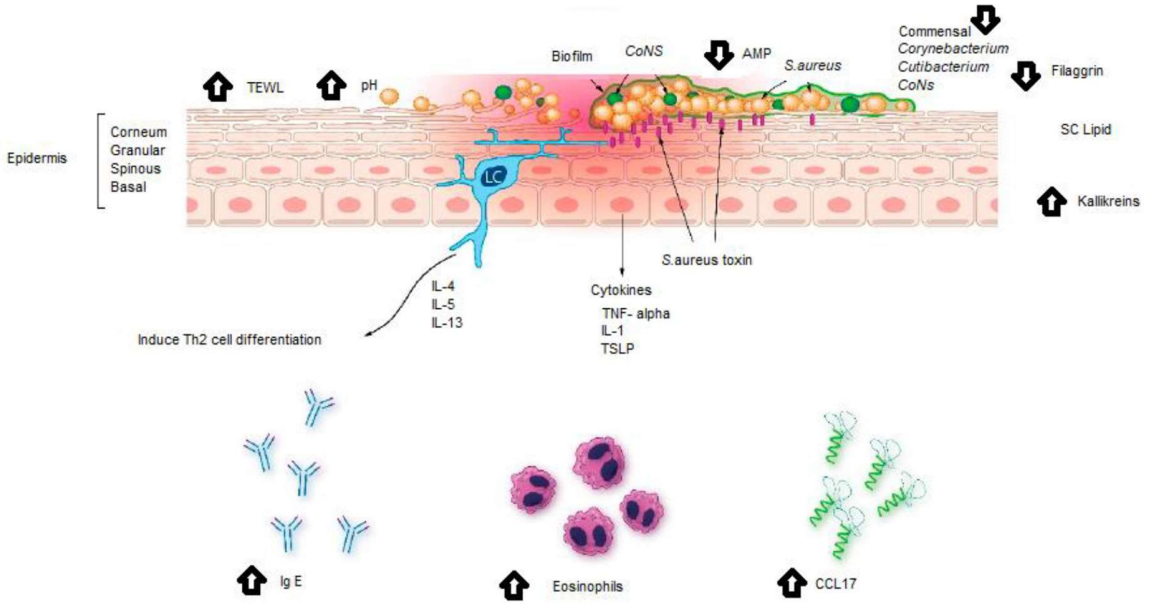

Figure 2. Skin barrier dysfunction. In AD there is a decrease in transepidermal water (TEWL), pH, IgE, activated cytokine (TARC / CCL17) and an increase in eosinophils. In addition, the lipid composition of filaggrin, the stratum corneum (SC) and the serine protease (kallikrein), allows colonization by S. aureus. However, the decrease in coagula-negative Staphylococcus (CoNS) and antimicrobial peptides (PAMP), causes S. aureus multiplication, forming biofilms. Source: Kim 2019

1.2.1. Dysfunction of the Immune System

In addition to the dysfunction of the epithelial barrier, there is also the dysfunction of the immune system, resulting from the increase in serum IgE (3,17,22,23).

The dysregulation of the immune system results from the complex interaction of environmental and genetic factors (23). However, it is not known a lot of information about changes in immunity and epithelial function, however Th2 (IL-13, IL-31, CCL17), Th22 (IL-22 and S100As) and Th1 (IFN-ʎ and CXCL10) were found in AD (17). Thus, the increase in Th2 cells causes an increase in the production of IL-4, IL-5 and IL-13, which can be the site of induction of IgE and eosinophils (3,17).

In addition, the immune system consists of several T helper lymphocytes responsible for maintaining balance. These differ in Th1 or Th2 according to the cytokines produced from the innate immune system. On the one hand, Th1 lymphocytes activate macrophages, producing pro-inflammatory cytokines, such as interleukins (IL-1,2,6,7) and tumor necrosis factor-α (TNF-α). On the other hand, Th2 lymphocytes are stimulated by antigens, consequently producing anti-inflammatory cytokines, such as IL-4,10, and IL-3 and the growth factor-β (TGF-β). These cells cause the transformation to IgE, responsible for the role of joy, eosinophilic activity and the production of mucus in the gastrointestinal tract (3).

After birth, the immune system is immature, with Th2 lymphocytes prevailing, which stimulates the production of IgE by β lymphocytes, and consequently there is a greater likelihood of allergic reactions and an increase in mast cell production. Then the immune system transforms Th2 to Th1 and Th3 lymphocytes, which are responsible for

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maintaining the balance of Th1 and Th2 functions by releasing mediators, such as TGF-β. The Th1 and Th3 lymphocytes have the ability to stimulate the production of IgA by B cells. In addition, IgA contributes to the protection of the gastrointestinal environment against pathogenic microorganisms and by cytokines, which reduce inflammatory processes and increase greater tolerance of organisms to the antigen. Thus, their increase can cause allergic (Th2) or autoimmune (Th1) diseases (3).

1.2.2. *Staphylococcos* aureus and Cutaneous Dysbiosis

*S.*aureus is a gram-positive cocci bacterium, which prevails on the skin and nasal cavities (24). There is an association of *S.*aureus with AD, that is, in the worsening of atopy, in food allergy, occurs the dysfunction of T cells with the decrease in PAMPs, and finally, the increase in IgE (8,25).

In addition, *S.*aureus produces α-toxin pores for the host cell, forming pores on the keratinocytes, and destroying the epithelial barrier. It is also responsible for producing proteases, whose activity is increased in places where Th2 cytokines are found and the absence of filaggrin. The Lipoteichoic acid (ATL) demonstrated that it can destroy the epidermal barrier, inhibiting filaggrin and loricrin, causing an increase in water and consequently greater exposure to antigens (15).

In addition, *S.*aureus is made up of molecules, whose pro-inflammatory mechanisms contribute to the arrival of AD. Protein A induces an inflammatory response in keratinocytes, through the link with the tumor necrosis factor 1 (TNFR1) receptor (15).

However, the toll-like receptor (TLR) 2 and 6, the pro-inflammatory staphylococcal lipoproteins induce the keratinocytes to produce TSLP, causing a self-perpetuating Th2 response (15).

However, *S.*aureus is responsible for releasing phenol-soluble modulins (PSMs), which stimulate inflammation with specific effects on receptors. A PSM induces keratinocytes to produce IL-36 causing an inflammation mediated by T cell. However, Th17 excretion occurs through IL-1 and inactive lymphoid cells. However, PSM δ (toxin-δ) stimulates dermal mast cells, and consequently skin inflammation (15).

Skin barrier dysbiosis (Figure 3.) is a characteristic of AD and the defects in the epidermal barrier promote the interaction of external antigens with immune cells resident in the skin, exacerbating local inflammation (26).

The severity of AD is associated with colonization by *S.* aureus, which is represented in the form of biofilm on the surface of the skin with AD. The biofilm of *S.* aureus increases rapidly causing hypoxia and damage to the protective epidermal barrier (26). In addition, filaggrin degradation products are responsible for pH regulation and hydration. *S.* aureus grows at a pH between 5 and 9, but acidification mediated by degradation products of filaggrin limits the growth rates and adherence of *S.* aureus to keratinocytes. Thus, the alkalinization caused by the decrease in filaggrin and its degradation products favors the proliferation, adhesion, biofilm formation and persistence of *S.* aureus on the skin of AD (26).

Thus, the exacerbated growth of *S.* aureus is directly related to the severity of AD, leading to refractory and recurrent infections, with greater resistance to the host's immune responses and less susceptibility to antimicrobials when compared to their planktonic equals (26). However, the improvement of AD is related to the decrease of *S.* aureus. Although the use of antibiotics and antiseptics can reduce it, recolonization often occurs in a few weeks, leading to limited clinical improvement and relapses of pathological manifestations (26).

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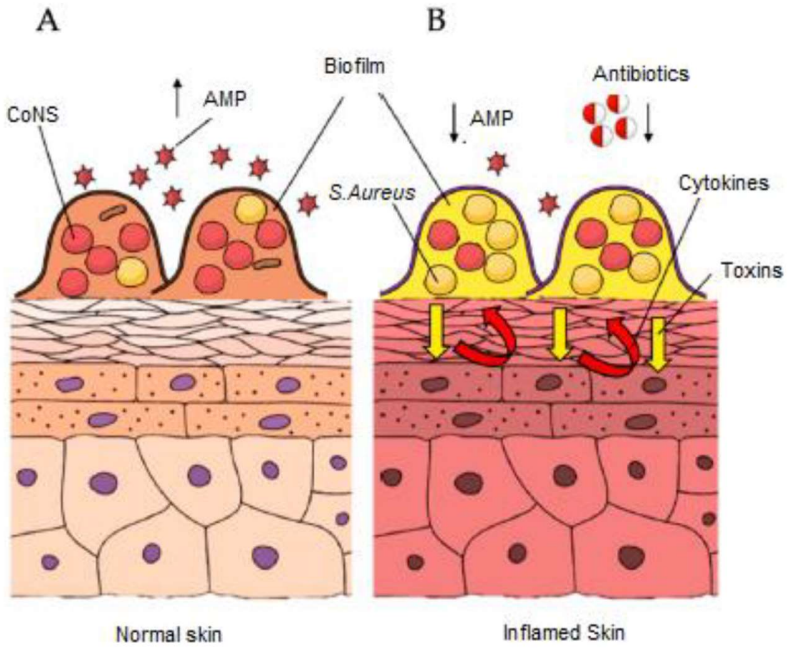

Figure.3 Cutaneous dysbiosis. (A) In normal skin homeostasis, coagulase-negative staphylococci (CoNS) compete with Staphylococcus aureus. (B) In dysbiosis and infection in AD, there is an overexcretion of inflammatory cytokines that promote the overgrowth of S. aureus. Source: Domenico et al. 2019

1.2.3. Cardiovascular Diseases and the Influence of Breast Milk on AD

The relationship between metabolic diseases (obesity and dyslipidemia) and AD can be mediated by chronic systemic inflammation, by pro-inflammatory cytokines, such as IL-6, tumor necrosis factor-α (TNF-α) and C-reactive protein (CRP), which increase oxidative stress and consequently there is an alteration in the expression of inflammatory genes (1). Thus, there is a correlation between the serum lactate dehydrogenase (LDH) level and AD, since the increase in LDH was due to the decay of inflammatory conditions induced in the skin (16).

However, breast milk in newborns, in addition to being a source of nutrition and hydration, also contains several immunologically active cells and molecules, such as immunoglobulins, lactoferrins, growth factors and cytokines (27). The cytokines found in breast milk are thymic stromal lymphopoietin (TSLP) and β growth factor (TGF-β). TSLP is responsible for maintaining Th2 responses and defending against helminth infections and in the pathogenesis of allergy-related diseases. Thus, TSLP is associated with AD and also with asthma. In addition, TSLP activates skin cells, increasing the Th2 response and interacting with cells returning to the skin to induce better production of IL-4, as this contributes to the maintenance of chronic AD inflammation. However, TSLP contains human isoforms of TGF-β (TGF-β1, TGF-β2, TGF-β3) that are responsible for inhibiting allergic inflammation. In this way, TSLP and TGF-β are charged in the acute and chronic phase of the disease associated with allergy, thus becoming potential mediators of the preventive effect. However, they need more studies to prove the preventive effect on AD (27).

1.3. Intestinal Microbiota and AD

The microbes associated with the human digestive tract are called intestinal microbiota (15). The intestinal microbiota is composed of 1014 microorganisms corresponding to 10 times the total number of human cells (19). These microorganisms are considered non-pathogenic and together with the host cells they protect against pathogenic microorganisms. However, these microorganisms are the main source of essential nutrients and vitamins. They are also responsible for obtaining energy from amino acids and short-chain fatty acids. Finally, they control the growth and differentiation of intestinal epithelial cells and the production of intestinal hormones (19).

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In addition, intestinal and skin microbiota composition, maternal diet during pregnancy, antibiotic treatment during pregnancy and early childhood, Western lifestyle with chronic exposure to allergens, increase the risk of allergic diseases and AD (1)(28). Thus, the microorganisms that colonize the intestine have functions in the health of the host, including the digestion of dietary components, production of metabolites (fatty acids, glycolipids and vitamins) and regulation of the immune system´s maintenance. Therefore, intestinal balance is important for host immune homeostasis (23).

Table 1. Prevalent Bacteria in the human intestinal tract. Source: Filipe and Machado 2018

Facultative Anaerobes		Obligate Anaerobes	
Lactobacilli	G^+	*Bifidobacterium*	G^+
Enterococci	G^+	*Clostridia*	G^+
Streptococci	G^+	*Eubacterium*	G^+
Staphylococci	G^+	*Bacteroides*	G^-
Enterobacterium	G^-	*Fusobacterium*	G^-

Legend: G+ - Gram positive; G- Gram-negative

At birth, the fetus' gastrointestinal tract (GIT) is sterile. However, after its birth there is a development of species of the intestinal microbiota until the period when the child reaches 2 to 3 years, that is, when the intestinal flora is considered adult (3,15). Thus, after its birth the neonatal microbiota develops due to the exposure of intrinsic and extrinsic factors, such as colonization by microorganisms that come from the mother through childbirth and the environment (3,15).

Several studies show that the type of labor influences the development of the intestinal microbiota. During normal labor, the maternal intestinal microbiota is transmitted to the newborn and during cesarean section this process does not occur, resulting in a decrease in anaerobic bacteria. Also, colostrum and breast milk are factors that influence the intestinal microbiota, as there is a variable secondary microbial load originating from the nipple, ducts and lactiferous. Finally, in children who receive breast milk there is an increase in the number of *Bifidobacterium* and *Lactobacilli* (Table 1), which represent more than 90% of the intestinal microbiota (3). Therefore, the development of the microbiota in the newborn is related to the function of the intestinal barrier, as it reflects on immune modulation. Therefore, continuous microbial stimulation of the microbiota is necessary for the immune system to mature in the intestinal mucosa. However, if this continuous stimulus does not exist, changes occur, as in enzymatic patterns and in the mucosal barrier, and decrease in the mucosal surface area. Also, the decrease in IgA, which has the ability to tolerate non-pathogenic antigens on mucosal surfaces (3).

However, throughout this systematic review, there was a decrease in the immune response of Th2 cells and an increase in Th1 cells, normalizing the regulatory T cells. There is also a normalization of regulatory T cells, restitution of the function of the barrier and mucosa, an increase in the diversity of the intestinal microflora, a reduction in fermentation products and a consequent decrease in *S*.aureus.

Consequently, the objective of this article is to study the use of probiotics in AD as a method of prevention, safety and efficacy, from the pre / post-natal period in infants and children or adults, with the most studied and effective probiotics that belong to the genera *Bifidobacterium* and *Lactobacillus*.

2. Treatment of AD

In the treatment of AD, the prevention based on skin care through the use of moisturizers and emollients is crucial. There are also other treatments, such as environmental control and the use of topical corticosteroids, topical calcineurin inhibitors and systemic immunosuppressive agents (11). However, this project studies the use of probiotics as a new therapeutic approach in AD.

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2.1. New Therapeutic Approach: The use of Probiotics in AD

Probiotics were introduced in 1907 by the Russian immunologist Elie Metchnikoff who suggested that lactic acid-producing bacteria in fermented milk could test human health benefits. In this way, new research has been initiated on its potential beneficial effects (3).

Probiotics, according to the Food and Agriculture Organization (FAO) and the World Health Organization (WHO), define probiotics as "live microorganisms that confer health benefits to the host when administered in adequate quantities" (8). Thus, probiotics have the ability to recover intestinal microflora and stimulate intestinal barrier function. This can affect the maturation of precursor cells of various tissues throughout the body (4). In addition, the World Allergy Organization - McMaster University Guidelines, Canada, considers that for the prevention of allergic diseases (GLAD-P) it is recommended the administration of probiotics to pregnant women with children at high risk of allergy, breastfeeding women with babies with high risk of allergy and babies who are at high risk, with a high focus on the prevention of AD, once that there is a high percentage of benefits shown in this disease (9).

In addition, the use of probiotics needs to meet certain criteria, such as: to be beneficial to health, presenting non-pathogenic properties; to be resistant to technological processing, presenting themselves as living cells and in adequate quantities; to resist the adverse conditions of the gastrointestinal tract, surviving the effects of hydrochloric acid and bile salts produced by the digestive system; to colonize the intestine, even if temporarily, by producing antimicrobial substances and having an effect on the immune system and metabolic activities (9).

So, the most common and studied probiotics belong to the genera *Bifidobacterium* and *Lactobacillus*. Other bacterial genera species such as *Bacillus*, *Enterococcus* and *Streptococcus*, in addition to the yeast *Saccharomyces*, are also classified as probiotics (19).

2.2. Probiotic: Mechanism of Action

Today, probiotics are an excellent therapeutic option in AD and other diseases. It produces beneficial effects, when administered in adequate quantities, having a role in the gastrointestinal tract and in the intestine-brain-skin axis (2,7,16). In addition, the benefits of probiotics come from several immunological mechanisms, the response to them is exacerbated in the atopic individual (9,11).

Probiotics have the function of modulating the activation of Th cells and the production of cytokines; induction of the response of regulatory T cells (Treg cell) and better recovery of barrier function (9). These act in the inhibition of Th2 cells and in IL (11). The decrease in Th2 cells, causes cytokines, such as IL-4; IL-5, IL-6, and IL-13, are not released and causes an INF-ʎ reduction (cytokine released by Th1 cells). Also, probiotics stimulate the secretion of IL-10 and the transforming growth factor β (TGF-β) (16). In addition, they can reduce inflammation, causing a reduction in cytokines, IL-4, IL-6; tumor necrosis factor-α, INF-ʎ and an increasing presence of IL-10 (1).

This way, there are several mechanisms of action (Figure 4.), where probiotics can act in atopy specifically in: restoration of Th1 or Th2 cytokines and in the increase of CD4 + Foxp3 Treg cells. Another underlying mechanism is the reduction in IgE and the increase in SCF. Finally, probiotics have the function of maintaining homeostasis and intestinal epithelial integrity, increasing antimicrobial production and consequently a reduced amount of pathogens (8).

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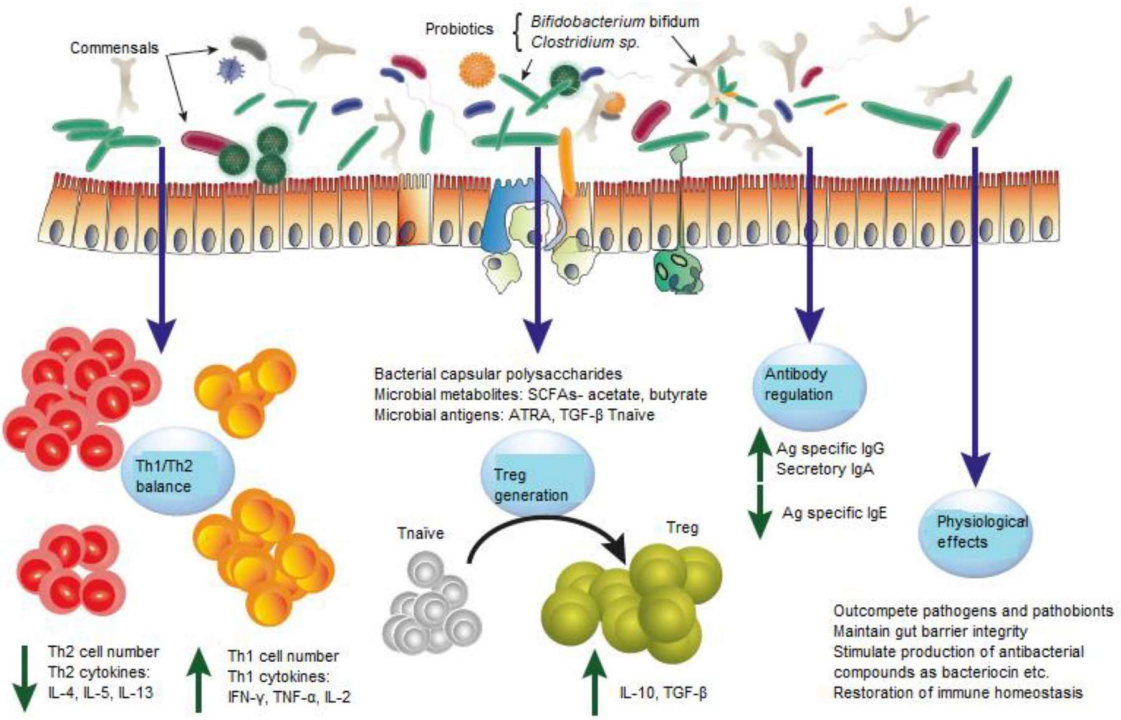

Figure 4. Probiotics´ mechanisms of action. Source: Sharma and Im 2018

2.3. Factors that Affect the Effectiveness of Probiotic

Heterogeneity affects the effectiveness of the probiotics use, influencing several factors (Figure 5.), such as: environmental factors, probiotics and the host. Environmental factors include maternal flora, type of labor, use of probiotics, environmental microbial load and eating habits. Some studies have correlated the type of labor with intestinal microbial dysbiosis, showing that caesarean deliveries promote the development of AD. Epidemiological studies have shown a decrease in the incidence of allergic diseases among children of farmers, when occurs the exposure to the uterus, with higher loads of bacterial endotoxin, a lipopolysaccharide, which is a protective factor. Probiotic factors depend on the dose of the probiotic, the number of foreign agents, and the time of prenatal or postpartum supplementation. And finally, the host factor that is made up of the basal intestinal microbial composition, genetic and environmental traits and the inherent immunological composition of the host (8,9).

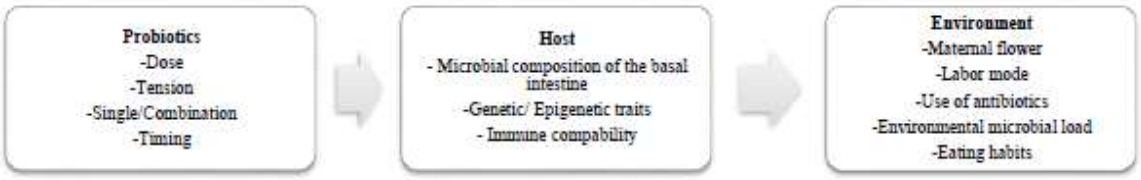

Figure 5. Factors that influence the effectiveness of probiotics in AD. Source: Dissanayake and Shimojo

2.4. Safety of Probiotics

Probiotics are generally considered to be safe, however they are not exempt from adverse effects (8,29,30). The Food and Drug Administration (FDA) classifies probiotics as food, cosmetics, dietary supplements, medical slides or medication on a case-by-case basis. Accordingly, the FDA considers that probiotics are not yet classified as drugs (29,30).

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Research on the clinical safety of probiotics before marketing is important to avoid unwanted effects. Therefore, probiotics must receive FDA approval, like any other drug, including filling out the application for investigation of new drugs (IND) and clinical trials, phases I-III (8). However, the labeling of probiotics by manufacturers may include unsupported therapeutic claims, such as incorrect identification of the probiotic and dose, subjecting the consumer to an error (29,30). Thus, the Health and Quality Research Agency (AHRQ) declares that the available literature is insufficient to determine the safety of the use of probiotics with confidence (30).

In addition, the WHO and FAO of the United Nations, demonstrate that probiotics have 4 adverse effects: systemic infections, harmful metabolic activities, excessive immune stimulation in susceptible individuals and gene transfer (8).

Therefore, future research should evaluate the efficacy and safety of probiotics, as there is a need for improved regulations and labeling. If probiotics and bacteriotherapy are approved as biotherapeutic, these investigations will open the way for more appropriate regulation and standardization in effective clinical use. However, the probiotics that are considered the safest and that dominate commercial formulas are *Lactobacillus* and *Bifidobacterium* (8,30).

Materials and Methods

This study was carried out at the School of Health Technology of Coimbra, being a systematic review. The study period ranged from 2014 to 2019 with a duration between September 2019 and June 2020. The study population was aged between 0 months and over 18 years, including pregnant women. In addition, all scientific articles and information were collected through the following databases, *PubMed*; *B-on* and; *Google Scholar*. Therefore, during the collection of information, it was made a detailed selection, with the consultation of the respective titles; abstracts with the following keywords: atopic dermatitis; probiotics and *Staphylococus* aureus.

This systematic review meets the mentioned inclusion criteria, that is, studies that mentioned all treatments used in Atopic Dermatitis, such as: articles published between 2014 and 2019; studies in humans aged 0 months and over 18 years, including pregnant women. Finally, studies that addressed the currently used treatments in AD. In addition, it fulfills the exclusion criteria, such as articles with a publication date greater than 2019. A total of 110 scientific articles were searched, with a progressive selection of 51 articles, in a final total of 32 articles and a thesis Master's Degree from the University of Fernando Pessoa, in order to meet all the inclusion and exclusion criteria mentioned above. However, the excluded articles were due to the following reasons: non-compliance with the selected study objectives; presentation of French language and violation of the study period defined for this review article. Thus, neither human, technical and financial resources nor dependent and independent variables are mentioned, once that it is a systematic review.

Results

Several studies have been carried out on the use of probiotics in AD and most of them are found to be effective in the treatment of this atopy, as a new therapeutic approach.

Lara and Moreira, in 2015, found that the immunomodulation exerted by these probiotics has a better response and less adverse effects than the application of corticosteroids. Table 2 shows positive results, where there is a lower incidence of AD in pediatric patients who used several bacteria. In addition, probiotic supplementation can accelerate the favorable evolution of atopy and cause children to remain asymptomatic for a period, even after the end of treatment (11). However, by analyzing Table 2, this also shows inclusive results in the use of several batteries analyzed, according to the authors Yang et al and Gore et al. (11).

Addor, in 2016, evaluated the tolerability and efficiency of the following probiotics: Lactobacillus acidophilus NCFM, *L*. rhamnosus HN001, *L*. paracasei Lpc-37 and *Bifidobacterium* lactis HN019, in children with AD aged between 6 months and 12 years. The severity assessment used the atopic dermatitis score (SCORAD). *Lactobacillus* rhmamnosus is a probiotic that has a modulating effect on the Th1 response; induces the synthesis of IL-10, has preventive action in the development of AD, as well as, the decrease of SCORAD in patients with AD. In Lactobacillus paracasei it was verified: the induction of IL-10, TNF-α and IFN-ʎ, modulating the immune response Th1 and Th2; increased activity

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of NK34 cells and stimulation of secretory IgA production. *Bifidobacterium* lactis also has an immunomodulatory effect on the lymphocyte response. Finally, Lactobacillus acidophilus potentiates the expression of cytokines that activate the Th1 response, promoting cell maturation and regulation of Th2 activity and inhibiting the growth of methicillin *Staphylococcus* aureus. Accordingly, we verified positive effects in the improvement of AD and absence of adverse effects, which guarantees the safe consumption of these probiotics (31).

Table 2. Results of the use of probiotics in AD in pediatrics. Source: Lara and Moreira 2015

Reference	Year	Type of Study	Sample	Species	Dosage	Result
Yang et al.	2013	Randomized double-blind placebo-controlled clinical trial.	100	*Lactobacillus* casei, *L.*rhamnosus, *L.*platarum and *Bifidobactéria* lactis	$5*10^9$UFC	Probbiotics heve sucess fully colonized the intestine, but it was not possible to find an additional therapeutic or immunomodulatory effect on AD treatment. ⬇
Hang et al.	2012	Randomized double-blind placebo-controlled clinical trial.	83	*Lactobacillus* plantarum CJLP133	$0.5*10^{10}$UFC	The results suggest that supplementation with probiotics *L.*plantarum CJLP133 is beneficial in the treatment of pediatric AD. ⬆
Gore et al.	2012	Randomized double-blind placebo-controlled clinical trial.	208	*Lactobacillus* paracasei CNCM I-2116 or *Bifidobactéria* lactis CNCM I-3446	10^{10}UFC	No benefit was found from early supplementation with *B.*lactis or *L.*paracasei, when administered as an adjunct to topical treatment of AD, nor was there an effect on disease progression up to 3 years of age. ⬇
Yavuz Yesilova et al.	2012	Randomized double-blind placebo-controlled clinical trial.	40	*Bifidobactéria* bifidum, *Lactobacillus* acidophilus, *Lactobacillus* casei and *Lactobacillus* salivarius	$2*10^9$UFC	The study proved to be effective in reducing SCORAD levels, showing the efficiency of the probiotics studied in AD. ⬆
Gerasimov et al.	2010	Ensaio clinico randomizado, dupla-ocultação e placebo-controlado	90	*Lactobacillus* acidophilus, DDS-1, *Bifidobactéria* latis UABLA-12 and fruto-oligossacáridos	$5*10^9$UFC	Evidence of significant clinical improvement in patients with AD and greater reduction in SCORAD scores in the intervention group. ⬆
Weston et al.	2005	Randomized double-blind placebo-controlled clinical trial.	53	*Lactobacillus* fermentum VRI-033 PCC	10^9UFC	There was na improvement in the extent and severity of AD in children with moderate or severe disease, with a marked reduction in the SCORAD score ⬆ ⬆
Rosenfeldt et al.	2003	Randomized double-blind placebo-controlled crossover study.	43	*Lactobacillus* rhamnosus 1907-2 and *Lactobacillus* reuteri DSM 12246	10^{10}UFC	After treatment, 56% of patients showed improvement in dermatitis while only 15% were part of the placebo.

Label: N- represents the patients who completed the follow-up of the respective study. DA- indicates atopic dermatitis, CFU- indicates colony-forming unit. Study favorable to the use of probiotics (p <0.05). Study inconclusive or without statistical significance (p > 0.05).

Simpson et al., in 2016, carried out a study with the participation of 415 pregnant women who were randomized to receive 250 mL per day of probiotic milk or placebo, from 36 weeks of gestation until 3 months after labor. Probiotic milk contained 5 × 1010 colony-forming units (CFUs) of *Lactobacillus* rhamnosus GG (LGG) and *Bifidobacterium* animalis subsp. lactis Bb-12 (Bb-12) and 5 ×109 CFU of *L.* acidophilus La-5 (La-5) per serving. The placebo was skim fermented milk, pasteurized after fermentation and it did not contain probiotic bacteria. In addition, TSLP and TGF-β (TGF-β1, TGF- β2 and TGF-β3) were analyzed using *ELISA* and *multiplex assays*, in breast milk samples collected 10 days and 3 months after labor of women participating in the Allergies Prevention Study for Children in Trondheim, (ProPACT), ProPact (n = 259). TSLP is responsible for potentiating the Th2 and the allergic type

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inflammation in the skin and lung. However, it has a regulatory function, because TSLP releases intestinal epithelial cells in response to commensal bacteria, which promote tolerogenic properties in dendritic cells (CD) and macrophages, that produce IL-10 and retinoic acid; promote regulatory differentiation of Treg cells. This way, the increase in TSLP observed in the probiotic group may encourage intestinal immune homeostasis, which is theoretically beneficial for the development of the neonatal immune system and prevention of AD. Finally, probiotics do not alter the concentrations of TGF-β breast milk (immunosuppressive cytokine) in 10 days or 3 months after delivery (27). It is also important to emphasize that the expansion and differentiation of Treg cells are promoted by the community probiotic and an environment rich in TGF-β created by probiotics (27).

Li et al., in 2018, found that supplementation with pre- and postnatal probiotics reduced the incidence of AD in babies and children. They also state that the treatment can be effective, even though there is a significant heterogeneity among the studies found. This can be explained by the fact that there are differences in the regions, at the time of supplementation and the various probiotic species selected. As an example: Asia, Europe, North America and Oceania, demonstrate that regional factors influence the overall results, due to the ethnicity and immunological mechanisms of the host that may be the key to different responses to probiotics according to the population and the geographic area (10).Thus, this article indicates that treatment with probiotics in early pregnancy and its continuity in the first 6 months of life may have a greater benefit in preventing AD. However, the prolonged use of probiotics during the postnatal period did not lead to a lower prevalence of AD in babies and children (10).

Reddel et al., in 2019, *Bifidobacterium* and *Lactobacillus* salivarius were evaluated in relation to intestinal microbiota (GM) modulation, to determine the persistence of probiotic bacteria by quantitative real-time PCR (qRT-PCR). The age range of the study population varies between 0 and 6 years, with diagnosis of AD. Users with AD showed a reduction in some bacteria which produce short-chain fatty acid (SCFA), such as *Bifidobacterium*, *Blautia, Coprococcus, Eubacteria* and *Propionibacterium*. The SCFAs have anti-inflammatory effects, through various mechanisms, such as epithelial integrity (preserving narrow junctions) and the maintenance of the mucus layer. The *Bifidobacterium* spp. has several benefits for human health, such as: the production of vitamins, stimulation of the immune system, inhibition of potentially pathogenic bacteria, improved digestion of food ingredients. In addition, several studies based on murine and in vitro models have highlighted the potential role of *Bifidobacterium* in reducing inflammation, inducing the production of anti-inflammatory cytokines and suppressing the Th2 immune response and the production of IgE. Thus, the absence of *Bifidobacterium* in children with AD is persistent with other studies, and may lead to a lack of anti-inflammatory effects (14).

Several studies have shown that the mixture of probiotics has more beneficial effects than a single probiotic (32). Fooland and Armstrong, in 2014, stated the effectiveness of the probiotic combination in AD. Dotterud et al. (2010) conducted a study to evaluate the effects of a probiotic mixture on the incidence of allergic diseases and allergic sensitization. Therefore, he investigated the possibility of a preventive effect in babies, regardless of family history of atopy. The mothers were supplemented during the pre and postnatal period, with a probiotic mixture composed of *L.* rhamnosus GG, *L.* acidophilus La-5 and *B.* animalis subsp. lactis Bb-12. Furthermore, the severity of AD was evaluated by SCORAD. This analyzed, that supplementation of mothers with this specific probiotic mixture was able to prevent, and not postpone AD in children at two years of age. In addition, they found that probiotics have anti-inflammatory properties, resulting in less epidermal penetration (4).

Notay in 2017, conducted a randomized clinical trial (RCT) using the combination of probiotics of *Lactobacillus* salivarius LS01 DSM 2275 and brief *Bifidobacterium* BR03 DSM 16604, in adults over the age of 18 years. Consequently, an improvement in AD and a reduction in plasma lipopolysaccharide (LPS) was determined. This is a marker of inflammation and permeability of the intestinal endothelium. This way, these researchers consider that the modified intestinal permeability has the consequence of immune activation dependent on the toll-like receptor (TLR). Therefore, a reduction in CD8 / CD38 / CD45RO T cell activation was analyzed in the probiotic group, which is considered a marker of immune activation (33).

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Study Limitations

There are some limitations about this review, however we hope it will be a useful tool for future investigations of the use of probiotics in AD. First, this study focused only on articles published in the English / Portuguese language, which can also exclude other eligible articles published in other languages. Secondly, the heterogeneity that influences the SCORAD values of several articles can also be a limitation. Finally, this review includes all age groups, but mostly newborns, children and pregnant women and less results from the use of probiotics in adults and the elderly.

Discussion

Generally, monotherapy with probiotics has positive effects in reducing the incidence of AD. However, the heterogeneity on several continents influences the result, which raises certain questions, such as: What is the best supplement that a person should take? Or what are the best conditions to live and to prevent a reduction in the incidence of AD? In addition, the relationship between the use of probiotics and the intestinal microbiota is remarkable, which induces the importance of maintaining the balance of epithelial integrity with the use of certain bacteria, such as short-chain fatty acid, which have an anti-inflammatory effect and consequently improvement of AD. Finally, there is a need for further study of TSLP and TGF-β to verify the relationship between intestinal immune homeostasis and TGF-β breast milk concentrations during pregnancy and after labor.

In addition, probiotic polytherapy may reduce the incidence of AD. The results induced the following question: What is the best effective therapeutic method, monotherapy or polytherapy for probiotics? Therefore, further studies are needed to verify the speed of efficacy, that is, an improvement in the decision regarding the combination of probiotics, especially in reducing the incidence of AD.

Conclusion

In this systematic review, it was found that the use of probiotics in the treatment of AD has beneficial effects and it can reduce its incidence. In addition, probiotics are generally considered safe, however they do have some adverse effects detected by the WHO and FAO of the United Nations, such as: systemic infections, harmful metabolic activities, excessive stimulation in susceptible individuals and gene transfer.
Therefore, probiotics and the combination of probiotics are future therapeutic strategies, which present potential and safety for the reduction of AD, once that several investigations have presented results with efficiency and effectiveness in reducing the incidence of atopy, presenting a decrease in SCORAD values. However, heterogeneity can influence the values, due to environmental, probiotic and host factors, in addition to other factors mentioned above. Also, the most studied and effective probiotics belong to the genera *Bifidobacterium* and *Lactobacillus* in the battle against atopy, since the pre / post-natal period in infants and children or adults.

As a future research perspective on this theme, it is crucial to study the evident anatomophysiology relationship of the intestine with the use of probiotics and their progressions in the incidence of AD. In addition, throughout this review it was important to emphasize answers to the following questions: What is the correct dosage of probiotics or the combination of probiotics and their adverse effects? When is the right time to administer probiotics? Does postnatal administration prevent AD in children? And finally, will it be crucial to expose children to microorganisms, in order to have greater resistance and consequently prevent AD? Consequently, further studies are needed to prove the use of probiotics as a new therapeutic strategy in AD. Finally, according to Hippocrates: "May your medicine be your food, and let your food be your medicine", which induces how the correct feeding of natural probiotics can have therapeutic benefits.

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