

Difficulties in Describing Allergic Disease Modulation by Pre-, Pro- and Synbiotics

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Abstract: The so-called hygiene hypothesis is, at least in part, accountable for the increase in allergic diseases in the developed countries. Although there is support for one of its primary predictions that host-microbe interactions in early life have longterm effects on the development of disease across populations, the theory has already proven to be imperfect as many more recent increases in certain diseases cannot be explained by the hygiene hypothesis. Nevertheless, many research groups are interested in the host-microbe interactions and are exploring the use of “live micro-organisms which, when administered in adequate amounts, confer a health benefit to the host” (probiotics) and “selectively fermented ingredients that result in specific changes, in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health” (prebiotics) to reduce the allergic disease onset or clinical outcomes. As the definitions of pre- and probiotics by itself were already adapted after their original dictation, it is not surprising that producing generalistic conclusions on the effectiveness of pre-, pro and synbiotic intervention in allergic diseases is very challenging as large differences exist in used species, methodologies, prebiotic(s) (mixtures) and probiotic strains. In this review we elucidate on the hurdles in describing prebiotics, probiotics and the combination being synbiotics in allergic manifestations.

Keywords: Prebiotics, probiotics, synbiotics, allergic diseases.

1. INTRODUCTION

The increase in allergic diseases in the developed countries is nowadays, at least in part, ascribed to improved hygiene and decreased exposure of the immature immune system to microbes in early life. The majority of publications on this so-called hygiene hypothesis indicate the imbalance between type 1 helper T (Th1) and type 2 helper T (Th2) cells as main mechanism behind the theory. Diseases that are dominated by Th1 cells are e.g. type 1 diabetes mellitus, Crohn's disease and multiple sclerosis, whereas asthma, eczema, and atopic dermatitis are Th2 driven diseases. Due to the Th2-dominant environment in the uterus to prevent rejection of the fetus, new-born babies have a Th2 dominant T cell repertoire that over time skews towards Th1 which has been seen as an indication for the maturing immune system [1]. Although there is support for one of its primary predictions that host-microbe interactions in early life have longterm effects on the development of disease across populations, the theory has already proven to be imperfect as many more recent increases in certain diseases cannot be explained by the hygiene hypothesis. Nevertheless, the condition within the uterus is considered to be essentially sterile, and new-born infants therefore begin to interact with microorganisms as soon as they come into the extrauterine environment. Within the first hours after birth, the gastro-intestinal (GI) tract is colonized by large numbers of mainly anaerobic bacteria predominantly originating from the mother's GI tract but also from the urogenital tract, skin, or the respiratory tract. The importance of the microbiota has been shown using germ-free mice which suffer from an impaired development of the mucosal immune system [1]. Several studies have investigated the possibility of restoring disbalances of the microbiota by oral ingestion of probiotics and/or dietary fibers (prebiotics) that selectively promote the growth of health promoting bacterial genus such as *Lactobacillus* or *Bifidobacterium*. In this review, the major objective is to show that producing generalistic conclusions on the effectiveness of pre-, pro and synbiotic intervention in allergic diseases is very challenging as large differences exist in used species, methodologies, prebiotic(s)

(mixtures) and probiotic strains. Therefore, Cochrane reports and meta-analyses are highly discussable regarding this topic, as will be discussed in detail below.

2. PROBIOTICS, PREBIOTICS AND SYNBIOTICS THE DEFINITIONS

As there are over 1000 different bacterial species residing in the GI tract, it is safe to say that the human host and the resident beneficial microbes are coexisting in a mutualistic system. The role of the microbiota within the GI tract became more clear in animal studies with germ-free mice since they show an impaired development of the mucosal and even systemic immune system. These data suggest that commensal colonizing bacteria are required to develop a properly educated and matured mucosal immune system [1, 2]. Furthermore, it has been shown that changes in the intestinal microbiota are correlated to allergic diseases [2-6], although the chicken and egg causality dilemma is not solved for this issue yet. As bifidobacteria are reported to be among the first colonizers of the GI tract directly after birth, reaching up to 99% of the fecal microbiota within 2 to 5 days after birth, this genus is considered to be of high importance for the developing immune system [6]. Vebø *et al.* have investigated the temporal development of a set of dominant resident microbes and correlated the individual profiles with the atopic measurements of participants to the KOALA study [4, 6]. They showed that there are significant phylum and genus level differences between sensitized and non-sensitized children. In addition, consistent age-specific colonization patterns appeared to be independent of the sensitization state. He *et al.* showed that non-atopic infants have a typical infantile bifidobacterial pattern that is dominated by *B. bifidum*, *B. breve* and *B. infantis*, whereas allergic infants harbored a more adult type of bifidobacteria dominated by *B. adolescentis* and some *B. infantis* [3]. Considering the presence of similar complex oligosaccharides in human milk ingested by both healthy and allergic individuals, it is interesting that only *B. infantis* is capable of releasing and utilizing monosaccharides from complex human milk oligosaccharides (HMOS), whereas *B. bifidum* can only release and *B. breve* can only ferment monosaccharides from HMOS [7]. Besides the differences regarding one of the major bacterial genus in the infantile microbiota, other studies have shown increased levels of potentially pathogenic clostridia, staphylococci and *Escherichia*

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coli in the intestinal microbiota of eczematous children [2, 5], whereas the streptococci and enterococci were significantly overexpressed in IgE sensitized infants [6]. He *et al.* also investigated the adhesion capacity of the different bifidobacteria as this is indicative for the suitability of the bacteria to attach to the mucus and thereafter colonizing the GI tract. The authors showed that bacterial species show varying adherence capacities to human mucus [3]. Although a substantial number of studies have been conducted on the differences in microbiota of allergic versus non-allergic children, little is known about the similarities or differences between the HMOS present in breast milk of allergic versus non-allergic mothers. Sjögren *et al.* showed that there was a large variation in the concentration of neutral oligosaccharides in colostrum that could not be explained by the allergic status of the mothers [8], but to our knowledge there is no literature on the acidic fractions or other specific characteristics like the presence of sialic acid.

Health-promoting bacterial strains, so-called probiotics, consume dietary fibers and, in theory, the combination of a probiotic and prebiotic, also called a synbiotic concept, may enhance the survival and function of probiotics in addition to resident beneficial microbes.

2.1. Probiotics

According to the FAO/WHO probiotics are “live microorganisms which, when administered in adequate amounts, confer a health benefit to the host” [9]. As these microorganisms are ingested, they should be able to resist the physicochemical stress of the digestive tract. The most commonly used probiotics belong to the genera of *Bifidobacterium* (mainly present in the colon) and *Lactobacillus* (mainly present in the ileum) and are differentiated from the microorganisms that constitute human commensal microbiota [10]. Besides the role of probiotics in the immune development they also play protective roles by directly blocking intestinal pathogenic microbes and enhancing mucosal integrity via epithelial cell stimulation [11]. Whether the probiotics should actually be alive in order to exert their protective role, remains to be elucidated.

2.2. Prebiotics

Human milk is considered to be the ideal nutrition for newborns as the quantity and quality of nutrients are adapted to e.g. the functional maturation of the GI tract. Human milk contains a.o. structurally diverse oligosaccharides reaching up to 10 g/L. These oligosaccharides can act as receptor analogs to inhibit the adhesion of pathogens on the epithelial surface [12, 13] and interact directly with immune cells possibly via specific sugar receptors [14]. The International Scientific Association for Probiotics and Prebiotics (ISAPP) recently reformulated the definition of prebiotics to “A dietary prebiotic is a selectively fermented ingredient that results in specific changes, in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health” [2]. Although this definition implies that prebiotics only work via the microbiota and do not have a direct influence on the host itself, evidence is now available that indicate direct effects of fermentable ingredients on the immune system (reviewed in [15]).

A variety of molecules can be considered as prebiotics, but the great majority is dietary fibers, such as the oligosaccharides inulin, galactooligosaccharides (GOS), fructo-oligosaccharides (FOS), sucrosyl oligosaccharides, acidic oligosaccharides (AOS; Fig. 1C) and xylooligosaccharides. The most widely studied have been inulin, GOS, FOS, a mixture of GOS and inulin and a mixture of short chain (sc)GOS Fig. (1A) and long chain (lc)FOS Fig. (1B) as they are resistant to *in vitro* digestion by pancreatic and brush-border enzymes. Chicory is the principal industrial source of inulin, from which FOS are often obtained by partial enzymatic hydrolysis. GOS are produced from lactose using beta-galactosidase [11]. Their

principal effect is related to the microbiota composition and activity. It has been shown that GOS, which are non-digestible oligosaccharides, can stimulate the growth of colonic bifidobacteria and, to a lesser extent, lactobacilli. GOS is therefore considered to be a prebiotic oligosaccharide [16, 17]. Oligo-saccharides cannot be digested by the host in the small intestine and are therefore available for fermentation by the colonic microbiota [18]. The fermentation of dietary fibers by the microbiota generates high amounts of short chain fatty acids, such as acetate, propionate, or butyrate, which constitute a source of energy and play a role of signaling molecule related to the immune system and metabolism [11, 18]. The immunomodulating character of these oligosaccharides is, o.a., shown in the Multicenter Infection Prevention Study (MIPS), in which full-breastfed, standard (placebo) formula and standard formula plus scGOS/lcFOS/pAOS have been administered to healthy infants during the first year of life. Although the overall infection incidence was lower than expected, the primary outcome parameter showed a reduced incidence of infectious episodes at 6 months of life. Besides an improved infection incidence, also the incidence of atopic dermatitis (AD) in the scGOS/lcFOS/pAOS was significantly lower than seen in the placebo group [19].

2.3. Synbiotics

The combination of probiotics and prebiotics is called “synbiotic”, which can have the advantage to promote the *in vivo* growth of the probiotic as well as similar resident strains in the GI tract [11, 14]. Human milk contains more diverse and complex oligosaccharides than those found in many animal milks [2, 13, 20, 21]. Furthermore, human milk contains the major phylotypes *Streptococcus* and *Staphylococcus*, and to a lesser extent *Serratia* and *Propionibacterium* [22]. Conversely, whereas previous work has identified potentially health-promoting *Lactobacillus* and *Bifidobacteria* as minor but common members of milk microbiota [23], very few sequences from these phylotypes were observed in milk samples investigated by Hunt *et al.* [22]. However, to our knowledge, there is no data available on the possible differences between the milk microbiota of allergic versus non-allergic mothers.

Depending on their enzymatic spectra, each bacterial genus or species will preferably use a specific substrate. Gopal *et al.* have shown that lactic acid bacteria have a preference for GOS with a low degree of polymerisation and are not able to digest complex HMOS [24]. Most strains belonging to the *Bifidobacterium* and lactic acid bacteria preferentially use fructans over glucose as a substrate. However, other bacteria such as Clostridia and Bacteroides are also able to grow on fructans. Nevertheless, their growth is less efficient than that of *Bifidobacterium* because of less-efficient substrate absorption (notably on oligofructose) [11]. To ensure that the probiotics will have enough energy source to survive in the GI tract, it might be beneficial to provide them with the proper prebiotic fibers. A synbiotic concept provides the appropriate fibers to a probiotic strain in order to promote the *in vivo* growth. The study of Schouten *et al.* showed that a dietary synbiotic concept of scGOS and lcFOS plus *B. breve* reduced the acute allergic skin response and anaphylactic scores to a larger extent than the sum of reduction caused by either the prebiotics or the probiotic intervention [25]. This suggests that besides the direct prebiotic effects for the resident microbiota and probiotics, the activation of both pathways somehow also enhance each other.

Although the populations of the microbiota generally differ between individuals, Arumugam *et al.* pose the existence of only three well-balanced host-microbial symbiotic states, called enterotypes, which are not nation or continent specific, and which are mostly driven by species composition [26]. As described above, differences in microbiota are correlated to allergic diseases. Getting more insight in this concept might provide a new strategy to

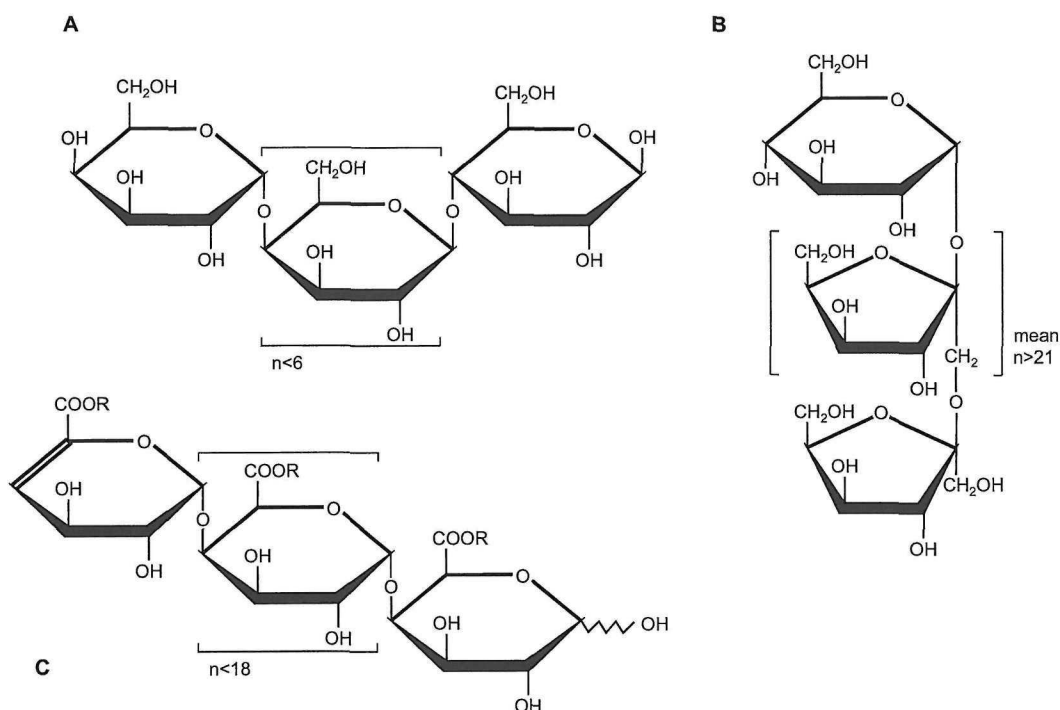


Fig. (1). Chemical structures of the non-digestible carbohydrates short-chain galacto-oligosaccharides (scGOS), long-chain fructo-oligosaccharides (lcFOS) and pectin-derived acidic oligosaccharides (pAOS).

A) Enzymatic elongation of galactose using β -galactosidase results in short-chain galacto-oligosaccharides (dp of 3 – 8). Structure represents two $\beta(1-4)$ -linked galactose molecules connected to a terminal glucose monomer. **B)** Long-chain fructo-oligosaccharides are produced from chicory inulin (dp of 7 – 60 and mean dp > 23). Structure represents one glucose molecule and two fructose molecules connected with a $\beta(2-1)$ -linkage. **C)** Basic chemical structure of pectin-derived acidic oligosaccharides consisting of $\alpha(1-4)$ -linked galacturonic acid molecules (dp of 1 – 20) ($R = \text{H}$ or $R = \text{CH}_3$) [53].

stimulate towards or away from the composition of that specific enterotype in order to reduce diseases like allergy. However, it also implies that the administration of one single probiotic strain might not be realistic to achieve prevention or treatment of a specific disease in all three enterotypes.

3. THE IMMUNE SYSTEM AND ALLERGIC DISEASES

The major function of the immune system is to defend the host from invading pathogens and other harmful insults. Pathogens that break through the mucosal surface barrier encounter two additional levels of defense, the innate and acquired immune responses. The efficient cross-talk between innate and acquired immunity enables the host defense to protect us from immune-related disorders and pathogenic invaders. The innate immune response is immediately activated after infection, while the acquired immune response takes at least several days for full development. It is therefore of key importance to discriminate “non-self” from “self” to avoid a destructive immune response against the host’s own tissue. The mucosal immune system is the largest immune component in the body and is of key importance in providing protection from the external environment. Although newborns have a competent immune system which is able to handle infections and responses to immunization, it invariably differs to the immune mechanisms present in adults. These differences are due to two main interrelated factors. The first is the development of the immune system; certain aspects of the innate and acquired immune system are not fully functional at birth and develop thereafter. The second is a consequence of the low exposure to antigen up to birth. Additionally, the immune system of newborns is biased against the production of pro-inflammatory cytokines, having high Th2 responses to prevent adverse immunological reactions between mother and fetus during pregnancy [14, 27]. The first postnatal year of life seems to be a key

period for programming the immune system in which feeding (i.e., human milk) and other factors to which a newborn is subjected (i.e., antibiotics) may have an influence on intestinal epithelial integrity and indigenous gut microbiota. From the moment solid foods are introduced and the dietary intake starts to resemble that of adults, both the microbiota and the intestinal immune system form important parts of our line of defense against ingested (pathogenic) microorganisms. In the gut, there is continuous cross-talk between microbes and the intestinal immune system and this requires a delicate integration of pro- and anti-inflammatory signals to regulate innate and adaptive immune responses and control inflammation [21].

In case of a disbalance in the immune system due to several different factors, immunological disorders can develop. Infants are more prone to develop Th2 disorders, with allergies and atopic dermatitis as most prevalent. Allergies, or hypersensitivity reactions, are classified into four different types by Coombs and Gell [28]. Aeroallergens, food allergens, drugs and insect venoms are the major allergens encountered that are responsible for induction of type I hypersensitivity reactions. This response may arise in individuals who have a propensity to exert an allergic response characterized by production of allergen-specific IgE. Binding of IgE to the high affinity receptor Fc ϵ R1 on mast cells followed by subsequent cross-linking of the receptors by the allergen provokes degranulation and the release of mediators such as histamine, leukotrienes and cytokines. The mechanisms by which allergen exposure through the epidermis can initiate systemic allergy and predispose individuals subsequently to atopic dermatitis, allergic rhinitis, and asthma, referred to as the atopic march, have become clearer in recent years [29]. About 60 % of food allergic patients have IgE-mediated allergic responses and IgE-mediated allergy is an important indicator of susceptibility to develop atopic diseases

like allergic asthma later in life [30, 31]. IgE mediated mast cell activation results in clinical symptoms involving the skin (urticaria, angioedema and atopic dermatitis), gastrointestinal tract (nausea, vomiting, abdominal pain, constipation, and diarrhea), airways (cough, wheeze) and systemic symptoms within a few minutes to one hour after ingestion of the food which can potentially lead to a life threatening anaphylactic shock.

4. ARE PREBIOTICS, PROBIOTICS AND SYNBIOTICS ABLE TO REDUCE ALLERGIC DISEASES?

Roberfroid *et al.* published an extensive review on the metabolic and health beneficial effects of prebiotics, in which it is stated that “a prebiotic effect exists” but one of the key questions remaining is “Which protocol(s) and methodology(ies) is (are) now available and validated to demonstrate links between a particular composition of the gut microbiota or a selective change therein and a particular physiological and/or pathophysiological well-being and health benefit?” [2]. As similar questions are frequently asked in case of probiotics and synbiotics, one might wonder whether the consideration of all prebiotics or probiotics as one common nominator is a reasonable approach [13, 16, 32]. Especially since the Food and Agricultural Organization in 1998 already stressed the importance of classification of dietary carbohydrates based on molecular size (degrees of polymerisation), type of linkage (α or non- α) and character of the individual monomers [13]. To our opinion, these biochemical specifications are needed to make a distinction between prebiotics to pinpoint which are indeed capable of health promoting activities and which are not. This was also shown in the study of Vos *et al.*, who reported that the specific combination of scGOS and lcFOS was significantly more effective in improving the vaccination response to an influenza-vaccine in a murine vaccination model compared to the single components or a mixture of scFOS with inulin [32].

Similar conclusions on the importance of classification were drawn in reviews on probiotic use in treatment of allergic diseases that showed that probiotics do not appear to be effective for the treatment of eczema. However, these same reviews also mention the significant heterogeneity between the studies that could be explained by the use of different probiotic strains, and that the pooled data can not exclude the possibility that a certain strain or combination of strains can still be effective [5, 33]. Moreover, functional foods based on the combination of two or more dietary fibers with different fermentability rates due to differences in chain lengths may result in a synergistic effect as the fibers are probably fermented in different parts of the GI tract [34]. Over the last years, many reports have indicated topics that should be taken into account when drawing conclusions about the effectiveness of prebiotic, probiotic or synbiotic intervention.

4.1. Methodological Differences

It is recognized that nutritional studies have a smaller effect size than seen in classical drug trials, which provides a relatively higher impact of confounding factors such as diet and lifestyle that may mask the exerted benefit [35]. Furthermore, Gourbeyre *et al.* stated that all of the studies regarding the influence of probiotics and/or prebiotics on allergic diseases performed so far were carried out under heterogeneous experimental conditions: different cohorts of children (with an allergic risk or not) and varying clinical protocols (different strains, doses, periods of exposure, and environmental conditions). They found it therefore difficult to compare the results of the numerous studies on the impact of these food supplements on allergy and suggested that future data should comprise homogeneous experimental protocols to compare results and to conclude any positive or negative effects [11]. Rijkers *et al.* also state that meta-analyses should not be done on data pools containing different (non-)efficacious probiotics, different conditions and different patient characteristics, as this will lead to an average non-effect due

to heterogeneity [35]. The study of Hougee *et al.* showed that selected bacterial strains show large differences in their ability to reduce allergic symptoms in a murine ovalbumin-induced allergic asthma model [36]. In the human situation, the contradicting study results of *Lactobacillus GG* administration to patients with eczema or atopic eczema are an example of the need for careful selection of data pools. The first studies showed a therapeutic effect for both conditions, whereas later studies showed only an effect in atopic eczema patients or no effect at all [37-42]. The authors of a review on these studies state that these differences might be due to protocol differences in the clinical set-up, but they also warn that it might be ascribed to the differences in probiotic preparations or formulations [43]. Similar recommendations are given by Rijkers *et al.*, as they pose that probiotic strains should not only be selected based on molecular typing, but also on metabolic characterization and metabolic consequences of interactions with matrix, background diet and host. In addition, metabolic capacities may be modulated by the growth conditions and the biological/physiological state of the probiotics [35], and should therefore, be taken into account.

As allergic diseases are usually divided into asthma, rhinitis, conjunctivitis, dermatitis, urticaria, food hypersensitivity and drug/venom hypersensitivity, one might think that defining the best outcome parameter for each disease is fairly simple. However, each of these allergic disease can be further subdivided, as e.g. asthma can be divided into non-allergic asthma and allergic asthma, of which the latter can be further splitted into IgE-mediated or non-IgE-mediated asthma. In case of dermatitis it can even be subdivided into 7 different disease terms [44]. It is therefore not surprising that the parameters and nomenclature of the investigated allergic diseases are not always one to one exchangeable [43]. An example that these different backgrounds of the disorders are important, is given by studies on atopic dermatitis in which the general study population did not benefit from synbiotic treatment, whereas the selective group of children that had IgE-associated atopic dermatitis (AD) did show a modest but significant reduction of AD [45]. To even further complicate study interpretations, different groups can use different markers for the same study parameter. A specific example is the effect of probiotics on cytokine responses in children with AD. Cytokine concentrations are measured in serum, plasma or after cultivation of the peripheral blood mononuclear cells (PBMC) of the children. Cultivation of PBMC is done in the presence of a specific stimulus like ConA or α CD3/ α CD28 or with an allergen-specific cow's milk protein to boost the cytokine production [45]. Some of these studies ascribe their effect to an up-regulation of regulatory cytokines and down-regulation of Th1 and Th2 cytokines, whereas another study shows up-regulation of regulatory cytokines and proinflammatory cytokines [45]. In addition, some studies scored pediatrician confirmed reduction of AD with or without anamneses, SCORAD or skin-prick tests, whereas others checked the prevention of AD with the same set of parameters [45]. Although the usage of the same parameters implies similar research questions, the distinction between reduction (i.e. treatment) and prevention of AD by probiotics should be very well described as they might function via completely different pathways and they will be investigated in a totally different study population. Furthermore, although fully in line with the atopic march, the majority of pre- and probiotic studies on eczema or atopic eczema are performed in infants/children, whereas asthma and allergic rhinitis are mainly studied in adults [43]. An example that early intervention in young infants with AD can lead to a subsequent reduction of asthma-like symptoms is given by van der Aa *et al.*, who studied the reduction of AD 12 weeks after administration of a synbiotic concept [46], and the long-term effects on asthma-like symptoms at 1 year after intervention [47]. They showed that the severity of AD after 12 weeks is significantly reduced in the synbiotic group compared to the control group, and the same groups also showed a significant difference on the reduction of asthma-like symptoms after 1 year.

One study showed a difference between the direct contact between PBMC and probiotic mixture *in vitro* and a situation in which the same probiotic mixture was administered via the GI tract. In the first situation, elevation of Th1 cytokines and regulatory IL-10 levels were observed, whereas oral administration resulted in a transient *ex vivo* immunological effect tending towards a diminished production of IL-10, TNF- α and IL-6. The authors conclude that extrapolation of *in vitro* results is not warranted in studies with probiotics [48]. Besides complications in extrapolation of *in vitro* to *ex vivo* results, also the extrapolation between host species is addressed in literature. Rodent (rat, mouse) microbiota differs from the human one. Therefore, the usually dominant Bifidobacteria in humans are increased by prebiotic administration while in rodents the Lactobacilli are promoted as a dominant component of the microbiota [11].

4.2. Defining the Proper Combination for a Synbiotic Intervention

A Cochrane Review published in 2007 aimed at determining the effect of different ingredients showing a prebiotic effect (GOS/FOS, FOS alone, GOS together with polydextrose and lactulose) on the prevention of allergic disease or food hypersensitivity in infants. According to the reviewers, only two randomized clinical trials of reasonable methodological quality reported outcomes related to allergic disease. The reviewers concluded that there is insufficient evidence to determine the role of prebiotic supplementation of infant formula for the prevention of allergic disease and food hypersensitivity [49]. Furthermore, according to Roberfroid *et al.*, only two dietary non-digestible oligosaccharides fulfill the criteria for prebiotic classification; galactans and inulin-type fructans. Only a limited number of randomized clinical trials evaluating the efficacy and safety of probiotics in a pediatric population are available, of which some are methodologically limited [2]. As the publication of Su *et al.* [49] has highlighted this issue already a couple of years ago, more research on the functional properties of the used prebiotics is desired as this gap has not been filled yet [35].

An even more serious challenge consists of defining the perfect combination of prebiotic(s) and probiotic strain(s) that can induce tolerance or reduce disease outcomes also by means of ideal doses and administration regimens. This necessity was also addressed by Su *et al.* [50], who administered several synbiotic combinations to mice, each containing one prebiotic (FOS, SOS, or inulin) and one probiotic (*Lactobacillus acidophilus*, *Bifidobacterium lactis*, or *Lactobacillus casei*). The authors then analyzed the amounts and survival of each probiotic strain in fecal samples. A SOS- or FOS-containing diet appeared to stimulate the growth of *L. acidophilus* and prolong its survival in the GI tract. FOS and inulin dietary treatment exerted the same effects on *B. lactis*. In addition, SOS, FOS, or inulin improved the survival and retention time of *L. casei* [50]. Similar effects have also been described in humans, as the administration of probiotic LGG in combination with GOS significantly increased the fecal concentration of probiotic *Lactobacillus rhamnosus* and commensal bifidobacteria as well as lactobacilli in feces from treated children compared to only LGG administration [51]. These data indicate that the prebiotic fiber that is used within a synbiotic concept should be checked for its suitability for the used probiotic strain. One way to check this is to perform an *in vitro* fermentation screening experiment with different prebiotics and probiotics as described for GOS [16]. Studies on GOS utilization by bacteria have proved that bacteria possess different abilities to ferment GOS. Individual strains have specific substrate preferences, probably because the differences in β -galactosidases specificity within each strain results in a differential preference for specific glycosidic linkage and/or the monosaccharide composition of the trisaccharides. These preferences determine the rate at which a microorganism can grow on a particular carbon source, because it will influence its ability to

compete with other bacteria in the colon, and the extent to which the substrate is converted into bacterial mass, as cell numbers will affect the degree of prebiotic and probiotic activity [16]. However, caution for determining the suitability of an energy source with an *in vitro* approach is warranted by the group of Grimoud *et al.*, who showed that *in vitro* growth of a variety of microorganisms on the carbon source resistant oligodextran was unsuccessful, whereas this glucooligosaccharide was significantly hydrolyzed *in vivo*. Although this example is a very specific one, this suggests that the lack of growth on resistant oligodextran could be due to the experimental conditions [52].

5. CONCLUSION

In conclusion, there are many variables that are making it very difficult to draw generalistic conclusions on the effectivity of pre-, pro-, and synbiotic interventions in allergic diseases. As the structural characteristics of prebiotics, biological/physiological state and growth conditions of the probiotics and the suitability of the prebiotic(s) to be combined with the chosen probiotic(s) in a synbiotic concept are not always fully described, properly designed study protocols should be assured in order to enable regulators to allow/recommend the promising nutritional based immunomodulating concepts based on solid scientific knowledge. Only when the exact same pre- and probiotics in the same allergic disease are investigated, definitive conclusions on the effectiveness of that pre-, pro-, or synbiotics on the prevention or treatment of allergic diseases can be drawn. It might therefore be necessary to omit the common nominators as prebiotics, probiotics and synbiotics and ascribe the immunomodulatory activities to describe the components in detail.

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CONFLICT OF INTEREST

None declared.

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