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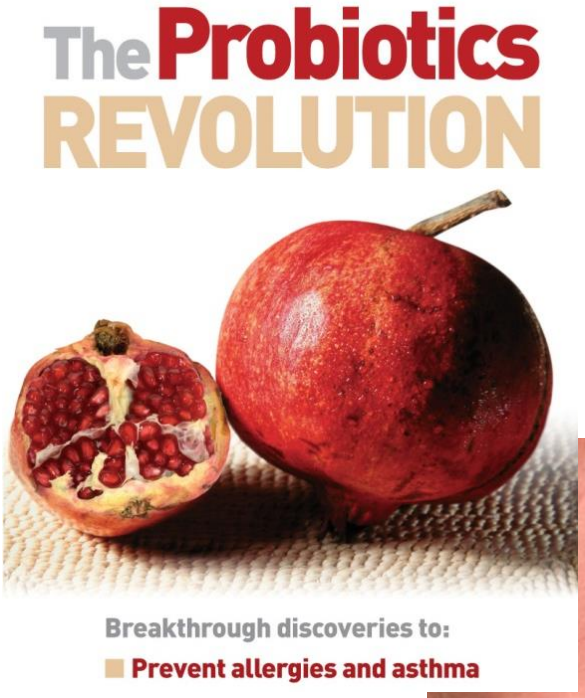

The Effect Of Probiotic Administration On The Risk Of Atopic Sensitization And Asthma induction In Children And Adults

Supervisors: Prof.dr.ir. D.J.J. Heederik
Dr. G. Doekes

Giannakou Christina, Student number: 3950654
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Abstract

Nowadays, atopic diseases and allergic asthma show high rates within the population and especially children. The underlying mechanisms of these diseases' induction, as unraveled up-to-date, present great heterogeneity. In addition, the environment and genetics are considered to play a greater or smaller role in their etiology. Immunomodulatory effects in the lung environment and allergy induction are associated with gut commensal microbiota and diet. Based on this association, antibiotic administration was used unsuccessfully to control microbiota composition in the past. Today, probiotic administration is considered a solution. It is also observed in children that the risk of atopic sensitization is reduced after administration of probiotics but no reduction on the risk of asthma. The underlying mechanisms and the possibility of the oral administration of probiotics as a factor of risk reduction against allergic sensitization are elusive. Different hurdles are raised in the way to establish probiotics as a mean of prevention against asthma or atopy induction, such as the administration protocol of probiotics and the strong dependence on the patient. The body of literature existing on this topic is growing but the data remain inconclusive and need further research. The aim of the present study is to review the existing data up-to-date on the effect of probiotics on asthma and atopic diseases.

Introduction

Nowadays allergic disorders affect around 20% of the population worldwide with an increasing prevalence (Warner *et al.* 2006), particularly in western countries (Cosmi *et al.* 2011), according to the recent study of World health organization (WHO) (Warner *et al.* 2006). Asthma is considered a common disease among children and the patients reach 235 million people worldwide (WHO). Allergies are considered to show a hereditary predisposition, since children that have members of their family suffering from allergy are found in higher risk in developing allergies compared to children that do not. Apart from genetics, environmental factors are also thought to play a very important role in the induction of allergy (Cosmi *et al.* 2011 and Penders *et al.* 2007); including diet, antibiotic use, surrounding environment rapid changes after post-industrialization (Hansel *et al.* 2013) and urban living which is followed by minimal contact to biodiversity (Hanski *et al.* 2012).

A role in the increasing prevalence of allergy has been claimed to play the reduced exposure to various microorganisms due to higher hygiene levels in early life having an impact on the host immune system, suggesting weakening of defense against certain diseases and easier development of autoimmune diseases. This hypothesis is termed as the "hygiene hypothesis" (Huffnagle 2010 and Toh *et al.* 2012). People today are getting in less contact with microorganisms due to the pasteurized or even sterilized food, medication against microorganisms as well as vaccination) and the status followed at the first stages of life starting form delivery (Ozdemir 2010). Since, early life stages contact of children with microbiota is correlated with immune maturity and decrease of allergy incidence (Ozdemir 2010 and Hörmannsperger *et al.* 2012). A neonate is colonized with microbiota at birth by contacting mother's commensal vaginal and fecal bacteria. It is observed that children born with caesarean section are colonized later with *Bifidobacteria* spp (*Bfdb*) and *Lactobacillus* spp (*Lcbs*) and develop more airway allergies (Ozdemir 2010). Another way microbiota is adapted already in the first week of life is breastfeeding and contact with the mother's skin and milk. Bfdb, *Bacteroids* (*Bact*), Lcbs and Lactic acid bacteria (Lac) are encountered on the mother's breast skin and oligosaccharides from breast milk helps bacterial expansion especially *Bfdb* (Ozdemir 2010 and Hörmannsperger *et al.* 2012). Viral infection in the lungs during the first years of a child's life is risky for allergic asthma induction (Hörmannsperger *et al.* 2012). It is also reported in literature that the children living on farms are exposed to a more diverse microbial environment and demonstrate lower rates of asthma (Ege et al. 2011).

Atopic diseases are characterized by chronic inflammation due to improper induction of Th2 type immunity triggered by exposure to specific environmental antigens and elevated antigen specific IgE levels in serum (Penders *et al.* 2007 and Toh *et al.* 2012). At present, atopic diseases are considered epidemic (Ozdemir 2010). Atopic disorders as well as asthma are diseases displaying great heterogeneity (Cosmi *et al.* 2011 and Penders *et al.* 2007). Asthma pathogenesis is considered to be allergic and non-allergic. Asthma heterogeneity is represented through distinct phenotypes and pathogenic mechanisms. Allergic asthma pathogenesis is thought to include mechanisms are dependent on Th2 cells (Trueba & Ritz 2013). Infections of the respiratory system are closely related with the asthma induction rates and there are suggestions that

psychological stress also can have an impact on development of asthma. However, the way stress can lead to asthma development, is still a subject under research (Trueba & Ritz 2013). Non-allergic forms of asthma are possible not to be induced by environmental factors, for instance air pollutants, viruses, and obesity. These forms of asthma are considered Th2 independent and require different therapeutic approaches. It is also observed, that different types of asthma could occur to one individual in his course of life (Cosmi *et al.* 2011). Th2-type responses and eosinophils contributing to asthma pathophysiology are based on several indications. Lately, an accumulating body of data suggests the contribution of Th17 in airway hyper-responsiveness induction, as reviewed by Cosmi *et al.* (Cosmi *et al.* 2011).

Exposure routes of allergens include also inhalation. The inhaled air contains particulates of different sizes. When particulates with diameters greater than 5 μm are inhaled, a part is deposited to the higher part of the respiratory system and another part is cleared through the GI tract. In this way, inhalation of these particulates results in oral and respiratory exposure (Heederik & Mutius von 2012 and Huffnagle 2010).

Several experimental and epidemiological data along with clinical observations point towards the change of the commensal microflora as an indicating contribution to allergic disease induction (Huffnagle 2010 and Toh *et al.* 2012). It is reported, that microbial-derived peptides and endotoxins have immunomodulatory action and possibly provide protection against allergy induction (Heederik & Mutius von 2012 and Hanski *et al.* 2012). Furthermore, it is supported in literature, that microbial biodiversity and atopy can be solidly correlated (Hanski *et al.* 2012 and Penders *et al.* 2007). In this frame, after the unsuccessful control of the microbiota composition by antibiotic administration, probiotic administration is considered a solution. (Hörmannsperger *et al.* 2012)

It is observed in children that the risk of atopic sensitization is reduced after administration of probiotics but no reduction on the risk of asthma. The underlying mechanisms and the possibility of the oral administration of probiotics as a factor of risk reduction against allergic sensitization are elusive. The body of literature existing on this topic is growing but the data remain inconclusive and need further research. The aim of the present study is to review the existing data up-to-date on the effect of probiotics on asthma and atopic diseases.

Mechanisms of asthma induction and atopy

The immune mechanisms leading to allergic diseases are still enigmatic and a large part of their etiology remains unknown (Michail 2009). Innate immunity effect on the adaptive immunity regarding tolerance induction is reported (Elazab *et al.* 2013 and Penders *et al.* 2007). Induction of dendritic cells (DCs) and initiation of T helper (Th) 1 immune responses is supported by Elazab *et al.* (Elazab *et al.* 2013) to play a central role in tolerance induction. Crucial role of T regulatory cells (Tregs), Toll-like receptors (TLRs) stimulation, tumor growth factor β (TGFβ) and interleukin (IL) 10 in immunosuppression mechanisms was also reported by Penders *et al* and Huffnagle (Huffnagle 2012 and Penders *et al.* 2007). In human and in mice $CD4^+$ Th cells are distinguished to effector and regulatory cells. The Th effector cells are separated to Th1 and Th2 cells (Cosmi *et*

al. 2011). The Th1 cells are considered to be part of the defense against intracellular pathogens including production of interferon γ (IFNγ) and phagocyte induction (Cosmi *et al.* 2011 and Trueba & Ritz 2013). Th1 cells are believed to participate in the induction of autoimmune diseases or chronic inflammatory disorders such as Crohn's disease (Cosmi *et al.* 2011). The Th2 cells are considered to participate in the host defense against helminthes, induce immunoglobulins, antibody production by B cells and produce cytokine; e.g. IL-4, IL-5, IL-9, and IL-13. Allergen-specific Th2 cells are thought to be substantially involved in the induction of atopic diseases (Cosmi *et al.* 2011 and Trueba & Ritz 2013) Th1 and Th2 cells are considered to interact through cytokine production. In addition, Th1 cells are considered able to inhibit Th2 cells' induction and vice versa (Trueba & Ritz 2013).

It was reported that, during development of the immune system exposure to a normal gut microbial flora allows the change of the balance Th1/Th2 in favor of Th1. However, in atopic diseases the balance is shifted to the Th2 response and increased production of immunoglobulin E (IgE) and induction of allergy (Elazab *et al.* 2013). The modulation of the immune system to Th2 responses was considered as a result of the composition of the gut microbiota. A recently growing a body of evidence points to Th17 allergen-specific cells, as a role holder in allergy and asthma (Lim *et al.* 2013). Albano et al. reported that the balance between Th17 and Treg cells is essential for immune homeostasis maintenance (Albano *et al.* 2013). Another aspect reported is the involvement of both Th2 and Th17 in allergic asthma. Th2 and Th17 differentiation is favored in the lung. Lim et al proposed that Th2 and Th17 are regulated in an antagonistic manner in the lungs (Lim *et al.* 2013).

Th17 lymphocytes are able of IL-17A, IL-17E (Bullens *et al.* 2013 and Albano *et al.* 2013), IL-8 (Cosmi *et al.* 2011), IL-21, IL-22, IL-26 (Bullens *et al.* 2013) production and induce the production of colony stimulatory factors (CSF) and CXCL8 by tissue resident cells. They express retinoic acid receptor-related orphan receptor (ROR) C and chemokine (C-C motif) receptor 6 (CCR6), too (Bullens *et al.* 2013). Production of IL-17A, IL-17E and IL-8 results in neutrophil recruitment. In this manner, Th17 cells contribute in inflammatory and various autoimmune diseases. Th17 cells also participate in host defense against extracellular pathogens (Cosmi *et al.* 2011). Different clinical studies support the role of Th17 in severe asthma pathogenesis. Th17 participation in severe asthma induction is also supported by the model developed by Song *et al*. (Song *et al.* 2012) IL-17 is found in lungs of humans with steroid-resistant asthma or severe asthma (Cosmi *et al.* 2011). Concerning asthma, the data that are available about Th17 and IL-17 from humans are limited (Bullens *et al.* 2013).

Human Th17 lymphocytes are considered to form a heterogeneous family. Their heterogeneity is evident by the many different hybrid cell types being reported, such as Th17/Th1, Th17/Treg and Th17/Tr1-like cells (Bullens *et al.* 2013). In patients suffering from allergic asthma another hybrid cell type, Th17/Th2, is described to be encountered more frequently than in healthy individuals. According to Cosmi *et al.*, presence of allergen-specific Th17/Th2 cells indicates their contribution in allergic asthma pathogenesis (Cosmi *et al.* 2011).

Furthermore, the immune pattern in pregnancy and asthma is also considered to be focused on Th17 and Tregs cells. Central role in peripheral tolerance are playing the Treg cells, which along with Th17 contribute to the course of pregnancy. The immune system of the mother during pregnancy has to face the challenge of tolerance towards the fetus. Asthmatic women face higher risk than healthy ones, since asthma can be a factor enhancing risks for gestational complication affecting the mother and the fetus. Among others preterm or cesarean delivery, preeclampsia, low birth weight and neonatal mortality are considered risks in asthmatic women during pregnancy. The ratio of Th17 and Treg cells in healthy pregnant women is lower than in the ones suffering from asthma, compared with the healthy non-pregnant women. Thus, the Th2 immune responses are altered during pregnancy (Toldi *et al.* 2011). Furthermore, in children with asthma and allergic rhinitis the IL-17A levels are also described as enhanced and Th17/Treg ratio is increased in moderate allergic asthma and rhinitis.

Equilibrium between Treg and Th17 cells seems to be essential to maintain immune tolerance, according to Albano 2013. Any change in either of the two cell types could lead to induction of atopic disorders, such as allergic asthma and rhinitis (Albano *et al.* 2013). Finally, it is a question if enhanced IL-17 levels and Th17 numbers occurring during allergic asthma and rhinitis hold protective role against lung inflammation or are the ones responsible for it (Bullens *et al.* 2013).

Airway and gut commensal microbiota

A huge number of microbes covers the surfaces of the body with the majority gathered in the gastrointestinal tract (GI) (Hörmannsperger *et al.* 2012 and Huffnagle 2010). The human gut lumen is colonized by microbes directly after delivery (Penders *et al.* 2007 and Ozdemir 2010). The microbial gut community shows great variation in species, with more than 500 different species, and different formation according to the gut part. For instance, the majority of bacteria in colon are obligatory anaerobes (e.g. Clostridium spp., Bfdb and eubacteria) and facultative anaerobes (e.g. Lactobacilli, Enterococci and Enterobacteriaceae). (Table 1) Gut microbiota, which principally maintains a complex symbiotic relationship with the host, has the ability to provide short-chain fatty acids, produce vitamin K and intake of ions along with maintenance of a crucial role in host immune well-being (Lebeer *et al.* 2010) as well protection against pathogenic bacteria (Penders *et al.* 2007). Commensal bacteria can be found free in the lumen or attached to the mucus, mucosal surfaces, food or feces forming biofilms and composing a diverse community. The forming of the biofilms can be influenced by many factors; dependent or independent on the host or the commensal bacteria (Penders *et al.* 2007).

Table 1: Bacterial genera in the human gastrointestinal (GI) tract.
(Penders *et al.* 2007)

Gram positive bacterial genera	**Gram negative bacterial genera**
Obligatory anaerobic	Obligatory anaerobic
Bifidobacteria Clostridia Eubacteria	Bacteroides Fusobacteria
Facultative anaerobic	Facultative anaerobic
Lactobacilli Enterococci Streptococci Staphylococci	Enterobacteria

As reported in the review of Penders *et al.*, the disturbance in the commensal microbiota during the first stages of life are linked to atopic disease causality, which seems not to be seriously affected by changes in the gut bacteria happening after early infancy (Penders *et al.* 2007). This is based on the emphasis given on the effect of the critical window period, when the immune system is not completely mature and tolerance against environmental agents is still formed, which was also shown in germ-free mice studies (Penders *et al.* 2007, Sudo *et al.* 1997 and Gaboriau-Routhiau 2003).

Recently, in human lungs was discovered the presence of microbes, in rather low concentrations though. However, is not yet clear if the community consists of constant members or ephemeral colonies (Huffnagle 2010, Penders 2007 and Marsland *et al.* 2013). High affinity of bronchial hyperreactivity and microbial diversity was found and higher correlation was reported for relatively 100 phylotypes. Families of Pseudomonadaceae and Comamonadaceae (Proteobacteria phylotype) were classified in the ones with great affinity to bronchial inflammation. These families include species able of being involved in asthma pathogenesis. These findings suggest that airway microbiota may also contribute to asthma pathogenesis through host-microbiome interactions (Huang & Lynch 2011). The lung microbiota though is distinctively different between healthy and non-healthy individuals (Marsland *et al.* 2013). Recent studies have shown that tolerance in airway and gut are closely connected (Gollwitzer & Marsland 2014). The commensal microbiota could have immunomodulatory effects on the lung environment directly or indirectly (Gollwitzer & Marsland 2014). Direct effect could come from the interaction between airway immune system and commensal bacteria in the lung (Gollwitzer & Marsland 2014). The indirect effect could be driven by the bacterial products and metabolites the intestinal microbiota produces, which reach the lungs via the bloodstream. Intestinal commensal bacteria can indirectly cause immune modulatory effects on the lungs by changing of DCs and T cells phenotypes (Figure 1) (Gollwitzer & Marsland 2014).

The gut is considered to be responsible for the detection of antigens inhaled and injected towards which tolerance is induced (Huffnagle 2010). Trompette et al., indicate that dietary fermentable fibers are participating in the control of inflammation in the respiratory system (Trompette *et al.* 2014*)*. The induction Th2 effector cells was reported to be decreased by DCs whose hematopoiesis in the bone marrow was augmented after fiber consumption (Trompette *et al.* 2014). Nevertheless, it is supported in literature that exposure to bacteria exposure in early

life and presence of airway microbiota could possibly be able of increasing or decreasing the risk of asthma induction (Beigelman *et al.* 2014).

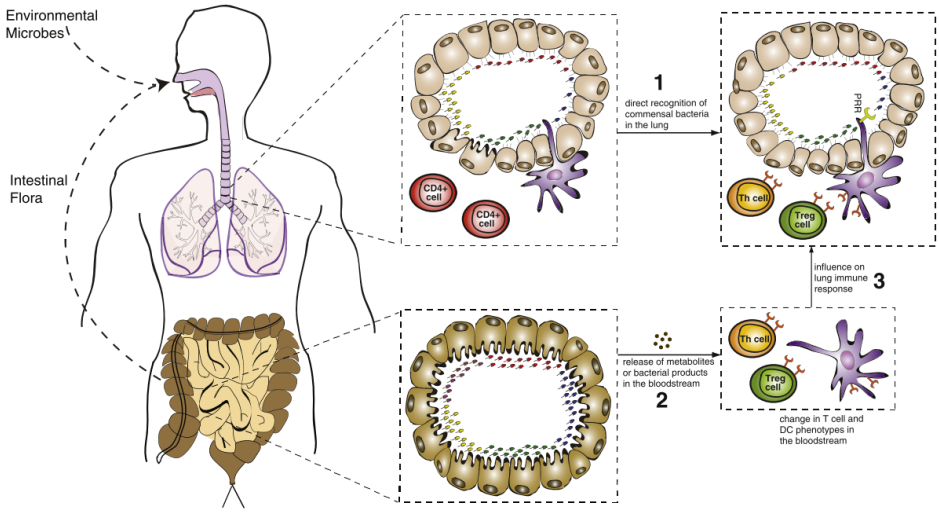

Figure1: Effect of microbiota on the immune modulation of the airway. There are indications that the commensal microbiota could directly or indirectly modulate the immune phenotype in the lung environment. Direct effect could come from the interaction between airway immune system and commensal bacteria in the lung. The indirect effect could be driven by the bacterial products and metabolites the intestinal microbiota produces, which reach the lungs via the bloodstream. Intestinal commensal bacteria can indirectly cause immune modulatory effects on the lungs by changing of DCs and T cells phenotypes. (Gollwitzer & Marsland 2014)

Professional and non-professional immune cells, like intestinal epithelium cells, DCs, T cells, B cells and macrophages, are able of recognizing and responding to microorganisms by pattern recognition receptors (PRRs). PRRs are receptors that recognize microorganism-associated molecular patterns (MAMPs) (Lebeer *et al.* 2010). MAMPs represent highly conserved microbial patterns with broad specificity (Lebeer *et al.* 2010 and Patten & Collett 2013). The cell surface TLRs and the intracellular nucleotide-binding oligomerization domain (NOD) consist two of the major groups of PRRs (Penders *et al.* 2007, Lebeer *et al.* 2010 and Patten & Collett 2013). TLRs are membrane glycoproteins located within plasma and endosomal membranes. TLRs are formed by three parts (Patten & Collett 2013). An extracellular MAMP-binding part with several leucine-rich repeats, a transmembrane part and a cytoplasmic signaling part, the Toll/IL-1R homology domain. The transmembrane domain crosses the cell membrane and also contributes in preserving the receptor in place. The last intracellular part of the TLRs carries out the intracellular transmission of the stimulatory signal (Patten & Collett 2013). Binding of TLRs with their respective ligands initiates activation of antimicrobial host defense, such as encoding of proinflammatory cytokines and chemokines (Figure 2 - Bermudez-Brito *et al.* 2012) (Patten & Collett 2013).The interaction of MAMPs-PRRs depends on several factors (Lebeer *et al.* 2010).

Table 2: Human TLRs and their ligands. Examples for certain TLRs and the ligand of the corresponding probiotic strains (Patten & Collett 2013).

TL receptors' location	Ligands	Examples of strains expressing TLR ligands
Plasma membrane		
TLR-2	Peptidoglycan	LTA (*Lactobacillus plantarum*) (Lebeer *et al.* 2010)
TLR-2/TLR-1	Lipoprotein Triachyl lipopeptides	
TLR-2/TLR-6	Lipotehoic acid	
TLR-4	Lipopolysaccharide	LPS (*Escherichia coli* Nissle 1917) (Lebeer *et al.* 2010)
TLR-5	Flagellin	Flagellin (*Escherichia coli* Nissle 1917) (Lebeer *et al.* 2010)
Plasma and endosomal membrane		
TLR-9	CpG-DNA	DNA from *Salmonella* and *Escherichia coli* strains (Ewaschuk *et al.* 2007)

Probiotics

People with airway allergy, such as rhinitis, are displaying alterations in the structure of the gut microbiota (Penders *et al.* 2007 and Huffnagle 2010). A body of growing data indicates that lifestyle, such as diet and antibiotic use, are able of changing the endogenous microbiota composition consolidating a new community (Penders *et al.* 2007 and Huffnagle 2010). The composition of microbiota was tried to be regulated with antibiotic administration but failed. Probiotics are considered to be a safe solution, but still many pieces of the puzzle need to come together in order to reach the full picture (Hörmannsperger *et al.* 2012). According to Joint FAO/WHO, probiotics are 'live microorganisms which when administered in adequate amounts confer a health benefit on the host' (Joint FAO/WHO). Probiotics are in offer today in different forms. They can be found as tablets and powders suggested for use by doctors or as dairy products and juices marketed by the production companies to the consumers (Verna & Lukcak 2010). The probiotic species mostly used today are members *Lactobacillus* or *Bifidobacterium* species (Lebeer *et al.* 2010). Positive effects were displayed after the use of LGG by children (Ozdemir 2010). Increased responses to certain vaccination and also an inhibition of airway infections were displayed (Ozdemir 2010). However, their use is quite limited due to the early stage of research. Adverse effects have been reported, following probiotic use. These adverse effects involve poor knowledge of host-microbiota interactions as well as allergic diseases' mechanisms of induction (Lebeer *et al.* 2010) There are still undetermined MAMPs (Lebeer *et al.* 2010) and structures that are able of modulating the host immune responses (Hörmannsperger *et al.* 2012). Side effects of probiotic administration were also recorded after clinical trials. A side effect recorded in clinical trials of administration of LGG supplements was sepsis (Ozdemir 2010). Enhancement of sensitization to allergens and negative effects on the GI are also considered and recorded side effects of probiotic use (Ozdemir 2010).

Cautious choice of probiotic administration during fetal status and first years of life result in a possible decrease of the total IgE levels and the risk of atopic disease's development as was shown by the meta-analysis conducted by Elazab et al. in 25 studies for 20 cohorts with 4031 participants (Elazab et al. 2013). However, the same is not true for asthma and wheeze (Elazab *et al.* 2013). A preventive role for probiotics is supported by clinical studies and research conducted on the mechanism of action of probiotics. Nevertheless, different strains have different influence on distinct patients. This variation in responses probably relies on the difference

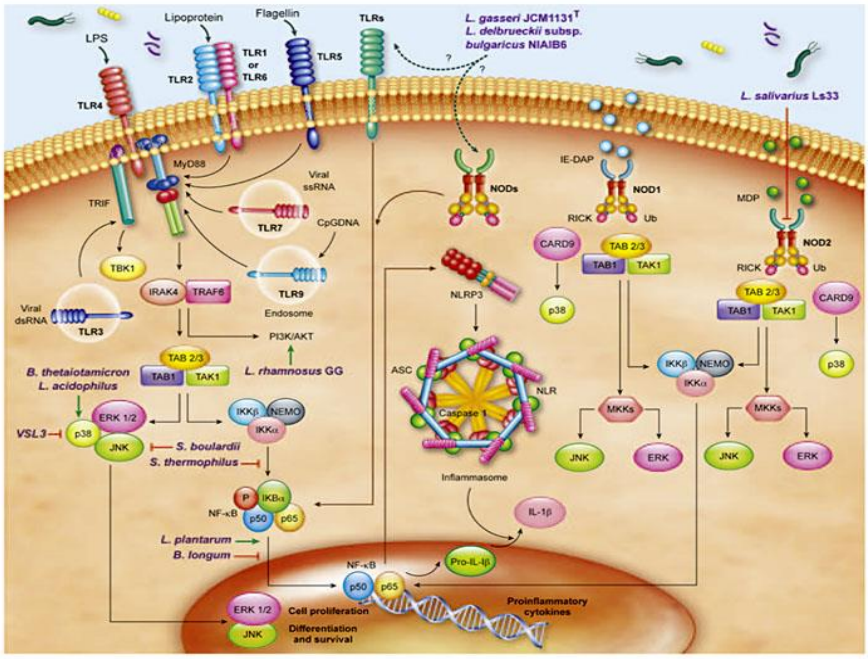

Figure 2: The intestinal microbiota interacts and communicates with the intestinal immune system via professional and non-proffesional immune cells. These interactions are performed by binding of the bacterial surface molecules, microbial-associated molecular patterns (MAMPs), to receptors on the plasma and endosomal membrane of the cells. The most studied group of these receptors are Toll-like receptors (TLRs). ASC = Apoptosis-associated speck-like protein containing a CARD; B. thetaiotamicron = Bacteroides thetaiotamicron; CARD9 = caspase recruitment domain-containing protein 9; ERK = extracellular regulated kinase; IE-DAP = D-gamma-glutamyl-meso-DAP; IKK = IκB kinase; IRAK4 = IL-1 receptor-associated kinase 4; JNK = Jun N-terminal kinase;MDP = muramyl dipeptide; MKK = mitogen-activated kinase kinase; NEMO = NF-κB essential modulator; TAB1/2/3 = TAK binding proteins; TAK1 = ubiquitin-dependent kinase of MKK and IKK; TBK1 = serine/threonine-protein kinase 1; TRAF6 = TNF receptor-associated factor 6; Ub = ubiquitin. (Bermudez-Brito *et al.* 2012)

between the commensal bacteria composition within population (Huffnagle 2010). It is a general belief that the possible influence of probiotics in risk of atopic sensitization could rely on the probiotic strain used (Elazab *et al.* 2013). Administration of Lactobacillus reuteri ATCC 23272, (Elazab *et al.* 2013) Lactobacillus rhamnosus GG, or Bifidobacterium lactis Bb-12 resulted in significantly reduced Airway inflammation and decreased allergen-specific IgE levels were displayed (Ozdemir 2010 and Elazab *et al.* 2013). Probiotics communicate with target cells through surface molecules. Gram-positive bacteria possess cell wall consisting mainly by a thick peptidoglycan layer decorated with proteins, teichoic acids and polysaccharides. Gram-negative probiotics, such as Escherichia coli strain Nissle 1917, possess cell wall consisting by a thin peptidoglycan layer, a periplasmic space and an outer membrane with lipopolysaccharide (LPS). The outer membrane is further decorated with proteins and polysaccharides. So, the different strain-specific effects and interactions with the host cells reported in literature can also rely on the variable alterations applied on the basic architecture of cell wall macromolecules (Lebeer *et al.* 2010 and Patten & Collett 2013).

Mechanisms of action of probiotics

According to Lebeer et al., three are the general mechanisms that the probiotics interact with the host promoting human health (Lebeer *et al.* 2010). The best studied mode of action until today is the ability of probiotics is their antagonistic character towards pathogens resulting in the

pathogens' exclusion or inhibition. Another mechanism is considered the probiotics' modulatory effect resulting in further development of intestinal epithelium. This modulatory effect can be translated into triggering of mucus and defensins production and modulation of tight junctions (TJ). The gut epithelial cells are connected to each other with intercellular connections known as TJ (Bergmann *et al.* 2013). The TJ was shown to loosen in people suffering from atopic disorders; including asthma (Hörmannsperger *et al.* 2012). Abnormal TJ formation is responsible for that, with increased epithelial permeability and higher risk of exposure to potential allergens and pathogens as a result (Trueba & Ritz 2013). What was also shown is that lung epithelium seems to loosen in people suffering from asthma (Bergmann *et al.* 2013).

The third mode of action refers to host immunomodulatory local and systemic responses. This effect presented encouraging results in *in vitro* and *in vivo* studies but not in clinical studies (Lebeer) However, the exact mechanism with which the probiotics interact with the host are not clear yet. A possible mechanism of action may involve alteration of toll-like receptors (TLRs) formation and proteoglycan recognition by intestinal epithelial cells (IEC) (Lebeer *et al.* 2012). As mentioned above, the interactions between probiotics and epithelial barrier in the gut are conducted through molecules on the bacterial surface, MAMPs, bound to TLRs and NODs (Penders *et al.* 2007 and Lebeer *et al.* 2012). Flagellin, lipopolysaccharide (LPS) and lipoteichoic acid (LTA) act as MAMPs. (Table 2) LPS and LTA produced by *Lactobacillus rhamnosus* GG (LGG) were reported to initiate production of cytokines after binding to TLR receptors (LPS to TLR4 and LTA to TLR2, Table 2).

Hoermannspreger et al. support that Th2 type immune responses are balanced through microbial products induction of Th1, Th17 and Tregs (Hörmannsperger *et al.* 2012). Clinical studies of probiotics administration to children with atopy resulted in up-regulation of IFNγ production, inhibition of IgE as well as of tumor necrosis factor-α (TNFα), IL-5, and IL-10 production induced by the antigens (Elazab *et al.* 2013). Nevertheless, it was reported that alterations in allergen-specific IgE and airway hypersensitivity were not correlated with the *Bifidobacterium longum* strain, AH1206, administration in mice, which comes in contrast to other studies (MacSharry *et al.* 2012). Penders et al (Penders *et al.* 2007) report Treg development due to *Lactobacilli* administration and *Bfdb* ability of immune modulation but the effect differs between the species administrated (Penders *et al.* 2007). In addition, in mouse model was reported enhancement of IFNγ and IL-10 production, inhibition of IL-4 (Th2 cytokine) and significant reduction on levels of OVA-specific IgE, IgA and IgG1 in serum (Ozdemir 2010 and Elazab *et al.* 2013). Inhalation studies in adult mice with *Lactobacillus reuteri* administration showed systematic enhancement of Tregs and suppression of the allergic response. Administration of an innocuous strain of *Escherichia coli* by inhalation led to long term prevention of allergic sensitization (Marsland *et al.* 2013).

Apart from the innate and adaptive immunity, more mechanisms may be responsible for induction of tolerance to microbial compounds by the host; including even neurological causes, such as stress. Stress is possible to affect directly or indirectly the immune system and in this way increase asthma exacerbations. A direct effect is considered the immune modulatory effect of

psychological stress on respiratory system and indirect effect can be regarded the enhancement of vulnerability of the airway towards infections (Huffnagle 2012 and Trueba & Ritz 2013).

Conclusion

Asthma and atopic diseases demonstrate high prevalence within the population and especially children, today. Many are considered to be the causes, inducing asthma and atopy sensitization. The underlying mechanisms of asthma and atopy induction, as unraveled until now, are found to demonstrate great heterogeneity. They compose a topic of intensive research but many pieces of the puzzle are still missing. Environmental and genetic factors are considered to play a greater or smaller role in their etiology. Gut commensal microbiota is associated with allergy induction. Diet is also associated with immunomodulatory effects in the lung environment. In the gut different microbiota composition or diet manipulated microbiota composition is linked to healthy or diseased state including also allergies. Today, a growing body of literature supports the existence of lung commensal microbiota (Trompette *et al.* 2014). There are also first indications of communication between lung and gut microbiota influencing immune responses. The data available to date, point also to the direction of combination of different airway microbiota composition and allergy. It is observed that there are certain strains present in majority in the lungs and also GI of patients suffering from atopy of allergy. A question still remains to be answered is if the microflora composition changes due to the allergy induction or triggers the allergic induction. Furthermore, from the information available today, no safe result can be made on the effect of a single bacterial species or the composition of the microbiota (intestinal and airway) is responsible for the above mentioned effects. Azad et al. reviewed five trials in children with follow-up to the age of 6 years old, without showing asthma as the primary outcome. Also, results from 3257 infants participated in nine randomized trials, pointed to risk ratio of 0.99 (95% confidence interval 0.81 to 1.21, I2=0%) of doctor diagnosed asthma to receive probiotics (Azad et al. 2013). In this review, it was concluded that randomized controlled trials did not demonstrate enough evidence to recommend probiotics as prevention against these disorders (Azad et al. 2013). However, also, combinations of strains or even combination of probiotics with prebiotics gave successful protection against risk of atopy. Fourteen randomized controlled trials evaluating different probiotics have been reviewed by Toh et al., where infants of families with a history of allergic disease with pre- and postnatal probiotic administration were participating (Toh et al. 2012). There it was reviewed that *L. rhamnosus* GG, *L. rhamnosus* LC705, *L. paracasei* F19, *B. breve* Bb99 and *Propionbacterium freuderechii* in combination with galacto-oligosaccharides showed positive preventive effects in clinical studies of eczema (Toh *et al.* 2012).

Probiotics are used in preclinical and clinical studies to investigate their preventive and treatment potential against asthma and atopy. Many are the obstacles still need to be overcome to establish probiotics as a mean of prevention of asthma or atopy induction. For instance, one of the problems faced, is the administration protocol of probiotics. The probiotic effect, as stated above is closely related with the patient. This shows the strong effect of the current microbiota

composition. So, personalized treatment and preventive policy is pointed to be the most optimal. However, further research will be needed to enlighten more aspects on the topic.

To sum up, taking into account all the above mentioned in this review, many points still need to be determined. The incomplete image of the asthma induction is an obstacle on the application of probiotics as a way to reduce risk of atopic disease development. In addition, research on probiotics and their interaction with the host is still in infant stages and further investigation at this direction will be needed.

Future perspectives

Different domains influence the research on the effect of probiotic administration on the risk of atopic sensitization and asthma in children and adults. The most important of them are summarized as, the complete understanding of asthma and atopy mechanism of induction, the mechanism of action of probiotics, the effect of the gut and airway microbiota to the host immune system and the communication between gut and lung microflora. In these growing fields of research, many questions still need to be answered.

Many are the problems faced, such as not certain definition and recognition of allergic diseases, due to many distinct phenotypes and patients' lifestyle. One of them is the different protocols used in different studies making it impossible to make comparisons (Lebeer *et al.* 2010 and Ozdemir 2010). Many aspects still vary and need to be optimized, including the time and duration of administration and the probiotic species used, as not everybody are benefited from the same microorganism. For instance, *Bifidobacterium* species displayed a safe profile after administration to neonates and infants, but not in immunosuppressed children (Ozdemir 2010). Furthermore, symbiotic (co-administration of probiotic and prebiotic) approaches are considered efficient in preventing allergic exacerbations (Gollwizer & Marsland 2014).

The species selection depends on the probiotic species forms and compound producing in combination to the gut microbiota composition of the individual treated (Lebeer *et al.* 2010 and Ozdemir 2010).This contrast is pointing to the importance of widely accepted administration protocol development, including the model organism used (MacSharry *et al.* 2012).

Moreover, concerning the fact that commensal bacteria and probiotics interact with the host immune system through ligands which differ depending the species but also even the strain, the identification of these ligands would give a boost to more targeted applications of probiotics. (Lebeer *et al.* 2010) According to Huffnagle et al., modulation of the commensal microbiota composition to enhance host mucosal immunity will result in encrypting the mechanism of probiotic action (Huffnagle 2012). However, further research on this direction will be needed to establish this aspect. In addition, according to Gollwitzer et al (Gollwitzer 2014) there are evidence supporting the contribution of airway microbiota to the disease exacerbation establishment of the gut-airway microbiota Bergman interactions will enlighten many aspects of the tolerance induction in the lungs and the effect of probiotics in asthma.

Finally, it is also essential to focus on the safety and risk assessment of the probiotic applications used to prevent development of atopic diseases. As well as, regulation should be developed for safe use of probiotics according to the up-to-date data available.

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