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# Probiotics and Mucosal Immune Response

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http://dx.doi.org/10.5772/50042

## 1. Introduction

There is complex and ubiquitous interface between the probiotic and resident bacteria (human microbiota) at various mucosal sites and the mucosal immune system. The probiotic bacteria are normally exogenous and transient as the resident bacterial communities of the human body are relatively constant companions of the human body and the mucosal immune system. This interface may result in local and systemic immune responses thus contributing for the preservation of the biological individuality of the human macroorganism.

## 2. Human microbiota

The human microbiota is an aggregate of microorganisms that reside on the surface and in deep layers of skin, in the saliva and oral mucosa, in the conjunctiva, the urogenital, to some extend the respiratory and above all the gastrointestinal tract. They include mostly Bacteria, but also some Fungi and Archaea. All these body parts are offering a relatively stable habitat for the resident bacteria: constant nutrient influx, constant temperature, redox potential and humidity. The skin flora does not interact directly with the mucosal immune system so it would be excluded from the present book chapter.

### 2.1. Oral microbiota

The oral cavity shelters a very diverse, abundant and complex microbial community. Oral bacteria have developed mechanisms to sense their environment and evade or modify the host. Bacteria occupy the ecological niche provided by both the tooth surface and gingival epithelium. A varied microbial flora is found in the oral cavity, and Streptococcal anaerobes inhabit the gingival crevice. The oral flora is involved in dental caries and periodontal disease, which affect about 80 %. of the population in the Western world. Anaerobes in the oral flora are responsible for many of the brain, face, and lung infections that are frequently

manifested by abscess formation. Oral bacteria include *Streptococci, Lactobacilli, Staphylococci, Corynebacteria* and various anaerobes in particular *Bacteroides*. The oral cavity of the new-born baby does not contain bacteria but rapidly becomes colonized with bacteria such as Streptococcus salivarius. With the appearance of the teeth during the first year colonization by *Streptococcus mutans* and *Streptococcus sanguinis* occurs as these organisms colonise the dental surface and gingiva. Other strains of streptococci adhere strongly to the gums and cheeks but not to the teeth. The gingival crevice area (supporting structures of the teeth) provides a habitat for a variety of anaerobic species. *Bacteroides* and *Spirochetes* colonize the mouth around puberty. However, a highly efficient innate host defense system constantly monitors the bacterial colonization and prevents bacterial invasion of human tissues. A dynamic equilibrium exists between dental plaque bacteria and the innate host defense system. [1, 2].

## 2.2. Respiratory microbiota

The nose, pharynx and trachea contain primarily those bacterial genera found in the normal oral cavity (for example, α-and β-hemolytic streptococci); however, anaerobes, *Staphylococci, Neisseriae* and *Diphtheroids* are also present. Potentially pathogenic organisms such as *Haemophilus, Mycoplasmas* and *Pneumococci* may also be found in the pharynx. Anaerobic organisms also are reported frequently. The upper respiratory tract is so often the site of initial colonization by pathogens (*Neisseria meningitides, C. diphtheriae, Bordetella pertussis*, etc.) and could be considered the first region of attack for such organisms. In contrast, the lower respiratory tract (small bronchi and alveoli) is usually sterile, because particles the size of bacteria do not readily reach it. If bacteria do reach these regions, they encounter host defense mechanisms, such as alveolar macrophages, that are not present in the pharynx [2].

## 2.3. Conjunctival microbiota

The conjunctiva harbors few or no organisms. *Haemophilus* and *Staphylococcus* are among the genera most often detected [2].

## 2.4. Urogenital microbiota

The urogenital flora is comprised mostly by the bacteria in the anterior urethra and the genital tract in women. In the anterior urethra of humans, *S. epidermidis*, enterococci, and diphtheroids are found frequently; *E. coli, Proteus*, and *Neisseria* (nonpathogenic species) are reported occasionally (10-30 %). The type of bacterial flora found in the vagina depends on the age, pH, and hormonal levels of the host. *Lactobacillus* spp. predominate in female infants (vaginal pH, approx. 5) during the first month of life. Glycogen secretion seems to cease from about I month of age to puberty. During this time, diphtheroids, *S. epidermidis*, streptococci, and *E. coli* predominate at a higher pH (approximately pH 7). At puberty, glycogen secretion resumes, the pH drops, and women acquire an adult flora in which *L. acidophilus, Corynebacteria, Peptostreptococci, Staphylococci, Streptococci* and *Bacteroides* predominate. After

menopause, pH again rises, less glycogen is secreted, and the flora returns to that found in prepubescent females. Yeasts (*Torulopsis* and *Candida*) are occasionally found in the vagina (10-30 % of women); these sometimes increase and cause vaginitis [2].

## 2.5. Intestinal microbiota

The number of bacteria in the digestive system alone is at least as big as the number of the stars in our home galaxy – the Milky Way as it contains no less than $10^{11}$ stars [3], thus forming a specific bacterial microcosmos the human gut. The number of bacteria increases in a logarithmic progression along the digestive system: the stomach ($10^1$-$10^3$ colony-forming units per milliliter (cfu/ml)), duodenum ($10^1$-$10^3$ cfu/ml), distal small intestine ($10^4$-$10^7$ cfu/ml) and above all the colon ($10^{11}$-$10^{12}$ cfu/ml). According to some authors the intestinal bacteria are forming the most densely populated ecosystem in the world [4]. The intestinal bacteria are really abundant when it comes to the various species and strains and their spatial distribution. The intestinal flora has a dynamic structure and is not isolated from the human host or the surrounding environment. There qualitative and quantitative variations in the gut flora depending on the diet, age, biotic and abiotic factors of the human environment, mucosal immune respose, presence or absence of organic disease of the host, intake of antibacterial medications, etc. The interface between the gut flora and the intestinal mucosal immune system is a perfect example for the interaction between the resident bacteria and the mucosal immune response. The gut flora is quite unique for each and every person and differs even in identical twins [5, 6]. The predominant bacterial genera and families inhabiting the human gut are presented on table 1 [4, 7-14]:

| Location | Facultative anaerobes | Gram staining | Obligate anaerobes | Gram staining |
|---|---|---|---|---|
| Duodenum and Jejunum | *Lactobacillus* *Streptococccus* *Enterobacteriaceae* | + + - | Solitary *Bacteroides* | - |
| Ileum | *Lactobacillus* *Streptococccus* *Enterococcus* *Enterobacteriaceae* | + + + - | *Bacteroides* *Clostridia* *Veillonella* | - + - |
| **Colon** | *Lactobacillus* *Streptococccus* *Enterococcus* *Enterobacteriaceae* | + + + - | *Bacteroides* *Bacillus* *Clostridium* *Fusobacterium* *Peptostreptococcus* *Bifidobacterium* *Eubacterium* *Ruminococcus* | - + + - + + + - |

**Table 1.** Predominant bacterial genera and families inhabiting the human intestine.

The intestinal flora may be divided to resident and transient. The resident bacteria can colonize and multiply successfully in the human gut for continuous periods of time as the transient microbial species can only do so for limited periods of time. The resident bacteria are able to adhere to specific molecules of the host or other adhesive bacterial species. Most of the transient bacteria are unable to do so or can only do it for a short time. The transient bacteria are usually ingested trough the mouth and belong to various genera and species [15].

## 3. Probiotic bacteria

The probiotic bacteria belong to the transient species as their presence in the human body is always a result of exogenous intake. There are numerous definitions for probiotics and they all correct in a way of their own. The concept for probiotics is constantly evolving, but essentially designates that they are "Living microorganisms which favorably influence the health of the host by improving the indigenous microflora". This definition was given by R. Fuller back in 1989 [16] and is very distinct from the one of the World Health Organization given in the beginning of the 21st century – "Live microorganisms which when administered in adequate amounts confer a health benefit on the host" [17]. There are also many other definitions and they all speak of the "whats", the "whos" and the "whens" but none speaks of the "hows". So if one would wish to include the "hows" it may sound like "Living microorganisms which when administered in adequate amounts may change the balance and keep the human body move in the right direction…". It does not say "favorable" as probiotics also have side effects and still it does not speak enough of "hows" so it can't really become the universal definition for probiotics. The intake of probiotic bacteria can be reviewed not only from a therapeutic and immunological angle but also unraveled throught the prism of ecology and cognitive philosophy.

The probiotic bacteria exert the unique quality to change the balance in a balanced way. They way they work is quite complex and fall pretty much into the witty remark of Albert Einstein "Life is like riding a bicycle – in order to keep your balance, you must keep moving" [18]. Indeed probiotic bacteria are alive and keep moving so as the human body. So when we want to understand probiotics everything comes to the balance between the outer and the inner cosmos of humans mediated by their mucosal surfaces.

The majority of commercially available probiotic bacteria belong to the genera *Lactobacillus* and *Bifidobacterium* but also strains of *E. coli*, *Streptococcus*, *Enterococcus* and even *Bacillus, Oxalobacter*, etc. Some yeasts are also being used as probiotics – *Saccharomyces*, etc. All commercially available probiotic bacteria must exert 5 crucial technological and clinical properties (fig. 1).

All these properties are equally important but the positive effect is by all means the most significant one:

- **Origin**: bacteria descending from the human gastrointestinal tract (GIT) (preferably);
- **Safety**: probiotic bacteria should be non-pathogenic and sensitive to the most commonly used antibiotics;
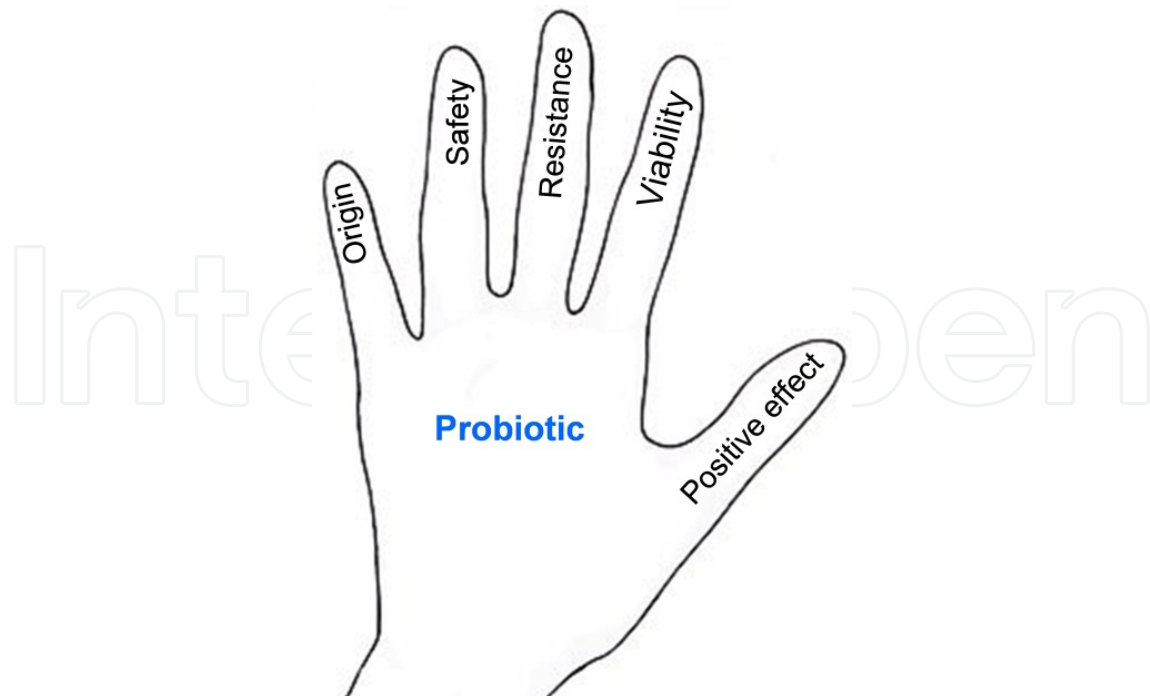
**Figure 1.** Main technological and clinical properties of the probiotic bacteria.

- **Resistance**: the bacterial strains should be able to survive the action of the stomach acid, the bile acids and the protease enzymes;
- **Viability**: these bacteria must survive the production process, proliferate in the small and/or large intestine, adhere to the gut epithelium and even colonize the small intestine and/or the colon for a finite time;
- **Positive effect**: their intake should be beneficial for health of the human macroorganism.

There is still conflicting evidence for the clinical efficacy of probiotic bacteria but yet they have been proven to be effective in infectious and antibiotic associated diarrhea [19, 20], urogenital infections [21, 22], immunologically mediated diseases such as inflammatory bowel disease (IBD) [23, 24] and atopic disease [25, 26], etc. Probiotic bacteria are being applied at various mucosal sites – orally, vaginally, as eye-drops, nasal sprays, etc. All mucosal sites are all connected in 3 different ways: anatomically, embryologically and most of all functionally.

## 4. Mucosal ecology

The intestinal flora is a specific blend of microorganisms, which have evolved and developed together with the macroorganism. These bacterial communities are highly variable and unique for all living persons. This is a result of time-limited migration of bacteria between humans in combination with their active interaction with the mucosal immune system, dietary and some genetic factors [27]. Human mucosal sites are classical habitats – they are normally populated by resident microorganisms. The human microbiota together with the mucosal surfaces of the human body form complex and dynamic ecosystems. All mucosal surfaces are directly exposed to the influence of environmental

factors of the outer world – they are all located at the edge of the outer world and the inner cosmos of the human body. The edge effect in ecology is the effect of the juxtaposition or placing side by side of contrasting environments on an ecosystem. The highest diversity of species and the strongest influence of the living creatures over habitats are found on edges [28]. The abrupt changes in the microbial community and/or the habitat may alter the balance and alter the the delicate equilibrium between the resident flora and human host – the so called homeostasis. The exogenous introduction of probiotic bacteria is unique as in terms of ecology it can be considered both as an abiotic environmental factor and a biotic factor of the living matter. The mucosal surfaces with their indigenous microbial communities are also unique as they are the combining the role of a habitat and a part of a living organism at the same time. The probiotic bacteria may interact with the resident flora and the microorganism and alter the homeostasis. The probiotic bacteria however interact with the mucosal immune system like any other bacteria.

## 5. Intestinal homeostasis

In healthy individuals there is a tolerance towards the resident flora. Because of that tolerance normally there is no aggressive cellular or humoral immune response towards the indigenous flora. The tolerance towards the intestinal flora and numerous dietary compounds is called oral tolerance. The oral and other types of antigen specific tolerance are dependent also on the mucosal permeability and the antigen clearance of *lamina propria*. This delicate equilibrium may be disturbed in various ways and lead to the development of an active disease. An example of such a disease is the IBD, in which the local and systemic immune response are aiming for the resident intestinal bacteria. The mucosal immune system in IBD is trying to permanently eliminate the intestinal microbiota, thus leading to the development of a chronic inflammation [29]. The mucosal immune system plays a key role for the maintenance of the mucosal homeostasis.

## 6. Mucosal immune response

The complex and well-set interaction between the probiotic bacteria, the indigenous flora and the mucosal surfaces are all possible because of the mucosal immune system and particularly the mucosa associated lymphoid tissues (MALTs). The MALTs are dispersed aggregates of nonencapsulated organized lymphoid tissue within the mucosa, which are associated with local immune responses at mucosal surfaces. Human MALTs consist mainly of the lymphoid structures within the GIT, urogenital tract, respiratory tract, nasal and oral cavities, the salivary and lacrimal glands, the inner ear, the synovia and the lactating mammary glands. The three major regions of MALTs are the gut-associated lymphoid tissue (GALT), bronchus-associated lymphoid tissue (BALT) and nasal-associated lymphoid tissue (NALT) however, conjunctiva-associated lymphoid tissue (CALT), lacrimal duct-associated (LDALT), larynx-associated (LALT) and salivary duct-associated lymphoid tissue (DALT) have also been described [30-34]. The organization of the MALTs is similar to that of lymph nodes with variable numbers of follicles (B-cell area), interfollicular areas (T-cell area), and

efferent lymphatics although afferent lymphatics are lacking. The overlying follicle associated epithelium is typically cuboidal with variable numbers of goblet cells and epithelial cells with either microvilli or numerous surface microfolds (M-cells). In addition, single lymphocytes can be observed within the epithelium, mucosa and *lamina propria*. All MALTs are morphologically similar although there are might be some differences in the percentage of T- and B-cells [35].

The GALT is typically organized into discrete lymphoid aggregates within the mucosa, submucosa and lamina propria of the small intestine called Peyer's patches (PP), the appendix, the mesenteric lymph nodes (MLN) and the solitary follicles. These aggregates are typically multiple lymphoid follicles with diffuse lymphatic tissue oriented towards the mucosa [36].

In the respiratory tract the NALT is the first site of contact for most airborne antigens and mostly presented by the tonsils and the adenoids at the entrance of the aerodigestive tract. The NALT bears certain similarities to the PP [34, 36].

The BALTs are organized aggregates of lymphocytes that are located within the bronchial submucosa. These aggregates are randomly distributed along the bronchial tract but are consistently present around the bifurcations of bronchi and bronchioli and always lie between an artery and a bronchus [34, 36].

The mucosal immune system has 3 main functions:

-    protects the mucosa against pathogenic microorganisms;
-    prevents the uptake of foreign proteins derived from ingested food, airborne matter and indigenous microbiota;
-    prevents the development of potentially detrimental immune response to these antigens in case they reach the body interior – i.e. oral tolerance in the gut.

In contrast with the systemic immunity, which functions in a sterile milieu and often responds vigorously to "invaders", the MALT protects the structures that are replete with foreign matter. The MALT must economically select appropriate effector mechanisms and regulate their intensity to avoid bystander tissue damage.

All MALTs have two basic structures: organized and diffuse lymphoid tissue. In the GALT the organized tissues are mainly the PP, MLN and the appendix as the diffuse ones are the intraepithelial lymphocytes (IEL). [37, 38]. The other MALTs are similarly organized.

The mucosal immune response has 2 phases:

-    inductive phase;
-    effector phase.

*Inductive phase*

The antigen uptake in the intestinal mucosa (especially particular antigens) occurs either through the specialized sampling system represented by the M-cells overlying the PP or across normal epithelium overlying the *lamina propria*. The M-cells may transport various

soluble antigens and even whole bacterial cells from the surface of the epithelium to the PP. Below the epithelium there are dendritic cells (DCs). The DCs perform phagocytosis of various antigens and present them to various immunocompetent cells in the mucosal immune system. The DCs may present the antigen to:

- T-lymphocytes in the PP;
- T-lymphocytes in the MLN – the antigen-loaded DCs may migrate from the PP through the afferent lymph vessels to the MLN and present the antigen there.

The cells, which present antigens are called antigen presenting cells (APC). Some MHC class II (+) enterocytes may also act as APC. The M-cells, DCs, PP and the MLN perform the antigen presentation and recognition, thus fulfilling the so called inductive phase of the immune response [39-41].

*Effector phase*

The diffuse lymphoid structures are mostly presented by the intraepithelial lymphocytes (IEL) – mature T-lymphocytes, and IgA producing plasma cells (activated B-cells). The T-lymphocytes are divided to CD4+ (helper or inducer) and CD8+ (suppressor or cytotoxic). In most cases the APC present the antigens to naïve CD4+ cells and activate them (fig. 2). The T-lymphocytes in lamina propria are predominantly CD4+, whereas the IEL are mostly CD8+. The activated CD4+ cells leave the organized lymphoid structures and using the lymphatic system reach the systemic circulation through the thoracic duct. The activated mucosal B-cells produce secretory IgA (sIgA), which is the principal mucosal immunoglobulin. Secretory IgA is a dimeric form of IgA and the two IgA molecules are binded by a joining chain. Secretory IgA inhibits the bacterial adhesion to the mucosa, carries out the lactoperoxidase and lactoferrin to the cell surface, takes part in the clearance of immune complexes and activates the alternative complement pathway. The IEL perform the effector phase of the immune response [37; 40].

The inductive and efector immune response are interdependent and sometimes overlapping.

The activated CD4+ may interact with other efector cells such as activated B-cells, CD8+ lymphocytes, etc. After priming, memory B- and T-cells migrate to other efector sites, followed by active proliferation, local induction of certain cytokines and production of secretory antibodies (IgA). The migration to other mucosal surfaces is called lymphocyte homing and it is possible because of the so called addressin receptors. By using the homing mechanism the lymphocytes sensitized in one part of the MALTs can reach all other mucosal sites [42]. About 80 % of the activated B-cells are found in the intestinal *lamina propria.* This is the main source of mucosal antibodies in MALTs [39; 43]. After priming, memory B- and T-cells migrate to effector sites, followed by active proliferation, local induction of certain cytokines and production of sIgA.

The intestinal epithelium and the GALT play a crucial role in the maintenance of the oral tolerance – antigen specific tolerance to orally ingested food and bacterial antigens [44]. All mucosal epithelial layers are a part of the innate immunity and serve as a first line of defense against numerous exogenous factors. The epithelial cells in the gut form a reliable

and highly selective barrier between the intraluminal content and the body interior. The disruption of this barrier could lead to the development of an inflammatory response. This would be a result of the direct interaction between the GALT and the intraluminal antigens. This has been confirmed in animal models – the mice with genetically determined alterations of the intestinal permeability are developing intestinal inflammation [45, 46]. Normally there is a constant interaction between the intestinal epithelium and GALT thus making possible the existence of the oral tolerance [47].

There is a complex relationship between the intestinal immune system and the resident and transient intestinal microbiota and it is crucial for the epithelial cells and the mucosal immune system to distinguish between pathogenic and non-pathogenic agents. Intestinal epithelial cells and some enteroendocrine cells are capable of detecting bacterial antigens and initiating and regulating both innate and adaptive immune responses. Signals from bacteria can be transmitted to adjacent immune cells such as macrophages, dendritic cells and lymphocytes through molecules expressed on the epithelial cell surface – the so called pattern-recognitioning receptors (PRRs). There are numerous PRRs: major histo-compatibility complex I and II molecules and Toll-like receptors (TLRs). TLRs alert the immune system to the presence of highly conserved microbial antigens called pathogen-associated molecular patterns (PAMPs). They are present on most microorganisms. Examples of PAMPs include lipopolysaccharides (LPS), peptidoglycan, flagellin, and microbial nucleic acids [4, 48-50]. This is exactly how probiotic bacteria interact with the mucosal immune system – by their PAMPs.

There are at least ten types of human TLRs. In humans, TLRs are expressed in most tissues, including myelomonocytic cells, dendritic cells and endothelial and epithelial cells. Interaction of TLRs and PAMPs results in activation of a complex intracellular signaling cascade, up-regulation of inflammatory genes, production of pro- and anti-inflammatory inflammatory cytokines and interferons, and recruitment of myeloid cells. It also stimulates expression of co-stimulatory molecules required to induce an adaptive immune response of APC [4, 50]. The colonic epithelium expresses mostly TLR3 but also TLR4, TLR5, and TLR7 [51], while cervical and vaginal epithelial cells have a higher expression of TLR1, TLR2, TLR3, TLR5 and TLR6 [52]. TLR4 recognises LPS [53, 54], a constituent of the cell wall of Gram-negative bacteria, while TLR2 reacts with a wider spectrum of bacterial products such as lipoproteins, peptidoglycans and lipoteichoic acid found both in Gram-positive and Gram-negative bacteria [55, 56].

There is another family of membrane-bound receptors for detection of proteins and they are different from the TLRs. They are called NOD-like receptors or nucleotide-binding domain, leucine-rich repeat containing proteins (NLRs). The best characterised NLRs are NOD1 and NOD2. NRLs are located in the cytoplasm and are involved in the detection of bacterial PAMPs that enter the mammalian cell. NRLs are especially important in tissues where TLRs are expressed at low levels [57]. This is the case in the epithelial cells of the GIT where the cells are in constant contact with the microbiota, and the expression of TLRs must be down-regulated in order to avoid over-stimulation and permanent activation. However, if these intestinal epithelial cells get infected with invasive bacteria or bacteria interacting directly with the plasma membrane, they will come into contact with NLRs and will activate some certain defense mechanisms [58]. NLRs are also involved in sensing other endogenous

warning signals which will result in the activation of inflammatory signalling pathways, such as nuclear factor-kappa B (NF-κB) and mitogen-activated protein kinases. Both NOD1 and NOD2 recognise peptidoglycan moieties found in bacteria. NOD1 can sense peptidoglycan moieties containing meso-diaminopimelic acid, which primarily are associated to gram-negative bacteria. NOD2 senses the muramyl dipeptide motif that can be found in a wider range of bacteria, including numerous probiotic bacteria [59, 60]. The ability of NRLs to regulate, for example, nuclear factor-kappa B (NF-κB) signalling and interleukin-1-beta (IL-1β) production, indicates that they are important for the pathogenesis of inflammatory human diseases, such as IBD and especially Crohn's disease.

NOD2 are expressed mostly by DCs, granulocytes, macrophages and Paneth cells, as the TNFα and IFNγ up-regulate the expression of NOD2 in epithelial cells in intestinal crypts [59, 61, 62]. The overall expression of NOD1 and NOD2 increases in inflammation [63, 64].

The microbiota alone can also predetermine the direction of this response with it's PAMPs and their interaction with human PRRs. The NLRs and TLRs play a crucial role in the regulation of the inflammatory response towards indigenous and transient microbiota. The synthesis of various pro- and anti-inflammatory cytokines and/or activation of NF-kB may alter the direction of the immune response – from inflammation to anergy.

The activation of the APC occurs after the binding of the PRRs with specific bacterial PAMPs. The types of PAMPs determine the selective activation of Th1, Th2, Th17 or Treg by the DCs (fig. 2).
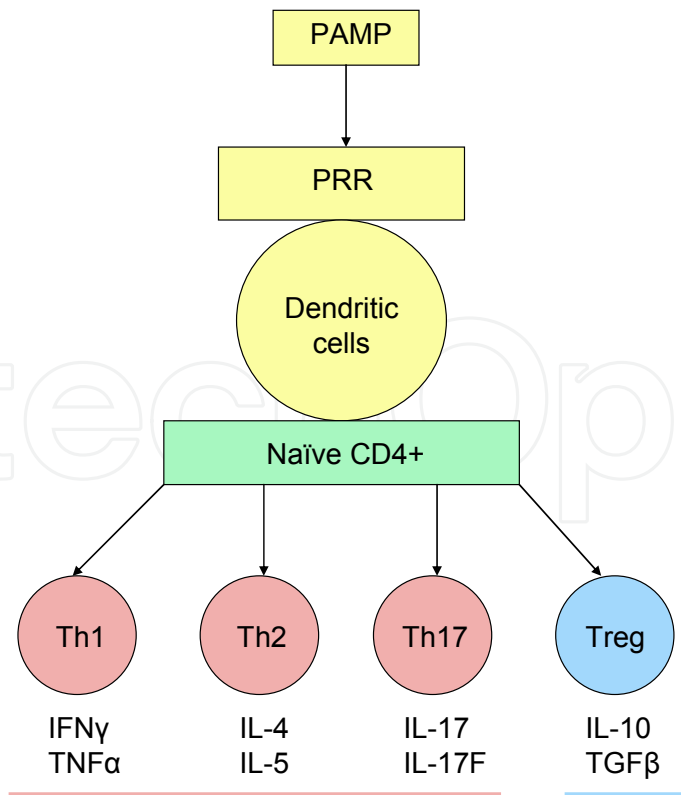


**Figure 2.** Interaction between the bacterial PAMPs, human PRRs, APCs, naïve CD4+ and activated CD4+ lymphocytes such as Th1, Th2, Th17 or Treg and their main cytokines.

The activated CD4+ lymphocytes may be divided in 2 groups:

- effector (Th1, Th2 and Th17);
- regulatory (Treg)

Effector CD4+ lymphocytes

- Th1-lymphocytes: they secrete IL-2, TNFα, IFNγ and GM-CSF. These lymphocytes take part mostly in the cell-mediated immune response, the normal functions of the macrophages and the delayed hypersensitivity reactions;
- Th2-lymphocytes: they secrete IL-4, IL-5, IL-6, IL-13 and mediate the humoral immune response, the synthesis of IgE and atopic disease;
- Th17-lymphocytes – some authors link them with the development of numerous autoimmune diseases. Their activation and functions are not fully studies and understood but they differ from the Th1- and Th2-lymphocytes. Their activation is mediated by TGF-β, IL-6, IL-21 and IL-23 but suppressed by IFNγ and IL-4. The Th17-lymphocytes secrete IL-17, IL-17F and IL-22.

Regulatory CD4+ lymphocytes

- Treg-lymphocytes: they secrete the anti-inflammatory IL-10 and TGFβ and mediate the intensity and the direction of the immune response. The animals with inborn deficiency of IL-10 and TGFβ develop acute enterocolitis with fatal consequences. This is a result of a paradoxical inflammatory response towards the resident intestinal flora [65-71];

There are parts of the indigenous microbiota that are less prone to induce inflammation, and there may even be bacterial genera with the ability to counteract inflammation. This seemingly inflammation-suppressing effect can be a result of different actions. The inflammation-suppressing fractions of the bacterial flora may be able to:

- counteract some of the inflammation-aggravating bacteria, which will decrease the inflammatory response;
- improve the barrier effect of the mucosa, which will inhibit the translocation of inflammation-inducing luminal contents into the body;
- directly interact with pro-inflammatory processes and cascades of the immune system.

All three actions may work simultaneously. Currently, the most studied inflammation-suppressing indigenous bacteria are certain species/strains of *Lactobacillus* and *Bifidobacterium*, and those are also the main bacteria used in the production of probiotics [72].

The inflammation alone can be a consequence of allergic reactions, infectious diseases and autoimmune diseases such as rheumatoid arthritis, diabetes type 1, multiple sclerosis and Crohn's disease, but a low-grade systemic inflammation also characterises the metabolic syndrome and the ageing human body. The long-term inflammation increases the risk for atherosclerosis, cancer, dementia and non-alcoholic fatty liver disease. Diabetes type 2 and obesity are also characterised by a low-grade inflammation but it is still unclear if the inflammation is the cause of the condition or just a result of it. The indigenous flora of the human body may trigger inflammation, and so favourable influence on the composition of

the indigenous microbiota can be a strategy to mitigate inflammation. The use of probiotic bacteria can affect the composition of the resident flora, but probiotics may also have more direct effects on the immune system and the permeability of the mucosa. The better the barrier effect of the mucosa the smaller the risk of translocation of pro-inflammatory components originating from the mucosal microbiota [72].

## 7. Probiotics and mucosal immune response in clinical practice

The polarization of the immune response is the reason why the oral intake of probiotic bacteria has been proven to be effective in allergic inflammation – atopic dermatitis, vernal keratoconjunctivitis but also in inflammatory bowel disease [23, 24]; infectious and antibiotic induced diarrhea [19, 20], urogenital infections [21, 22], atopic disease [25, 26]. Probiotic-induced immune modulation at mucosal sites distant from the gut supports the 'hygiene theory' of allergy development [73]. The 'hygiene theory' links the recent increase in the prevalence of allergic disease with modern western lifestyle, through altered patterns of gut colonisation characterised by a skewing towards an IFN-$\gamma$ mucosal cytokine response [74]. In addition some authors suggest that probiotics may have a place as adjunctive treatment in *H. pylori* infections and possibly in their prophylaxis [75].

Based on the clinical evidence we could assume that the effects of probiotic bacteria over the mucosal immune response may be divided into local and systemic. Indeed the efficacy of probiotic bacteria in atopic disease speaks of some systemic effect. Another perfect example for potential systemic efficacy are the immunological changes in breast milk, occurring after oral intake of *Lactobacillus bulgaricus* - "I. Bogdanov patent strain tumoronecroticance B-51" - ATCC 21815 [76]. According to the authors this is possible because of the functional entero-mammaric link and the functional redistribution of activated lymphocytes from the gut to the mammary gland and vice versa. In addition to this Dalmasso et al. [77] reported a novel biological property of probiotic bacteria: their capacity to affect immune cell redistribution by improving the competence of lymphatic endothelial cells to trap T lymphocytes.

The facilitation of oral tolerance and innocent bystander suppression by probiotic bacteria [78, 79] support the fact that particular probiotics not only drive protection against infection throughout the mucosal immune system, but also regulate the effector response. It is likely that different bacterial species operate through different mechanisms, indicating the importance of screening assays when identifying new isolates for clinical testing. It is suggested that a new term '*immunobiotics*', identifying those bacteria that promote health through activation of the mucosal immune apparatus, is a necessary evolutionary step as the foundation of our knowledge expand regarding the host–parasite relationships and their outcomes, as they relate to health and disease. Recognition of bacteria that promote mucosal T-cell function as '*immunobiotics*' moves probiotic biology forward by focusing on a mechanism of outcome, i.e. immunomodulation at distant mucosal sites. The human understanding of the interaction between the '*immunobiotic*' bacteria with the MALTs increases further and particular effector molecules and their receptor targets are being identified. A new focus in biotherapy can be expected to evolve. It still remains to convert

predictable shifts in mucosal immunity into practical health gains for the benefits of immunobiotic therapy to be realised [74].

## 8. Conclusion

The Roman Emperor and Stoic Philosopher Marcus Aurelius has said "Constantly regard the universe as one living being, having one substance and one soul; and observe how all things have reference to one perception, the perception of this one living being; and how all things act with one movement; and how all things are the cooperating causes of all things which exist; observe too the continuous spinning of the thread and the contexture of the web." [80]. Indeed the probiotics, the resident flora and the mucosal immune system are extremely strongly related and act as a single equilibrium and should always be investigated and described together. There is a long way to go until we fully understand and manage to control the interaction between the probiotic bacteria and the mucosal immune system.

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## Acknowledgement

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