CHAPTER-II

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2. Review of Literature:

The barrier of gastrointestinal tract keeps functioning against antigens from microorganisms and food. The establishment of indigenous microflora is the reason for the generation of immune-physiologic regulation within the gut. This has led to the introduction of novel therapeutic interventions based on the consumption of cultures of beneficial live microorganisms that act as probiotics.

Among the possible tools of probiotic therapy is advancement of a non-immunologic gut defense barrier, which embraces the normalization of increased intestinal permeability and transformed gut micro-ecology. Another possible mechanism of probiotic therapy is upgrading of the intestine's immunologic barrier, chiefly through intestinal immunoglobulin A (IgA) responses and mitigation of intestinal inflammatory responses, which creates a gut-stabilizing effect. Many probiotic effects are mediated through immune regulation, particularly through balance control of pro-inflammatory and anti-inflammatory cytokines. Data from various studies show that probiotics can be used as innovative tools to ease intestinal inflammation, calm down gut mucosal dysfunction, and down-regulate hypersensitivity reactions. Recent data indicates that differences exist in the immunomodulatory effects of candidate probiotic bacteria. Moreover, distinct regulatory effects have been detected in healthy subjects and in patients with inflammatory diseases. These results suggest that specific immunomodulatory properties of probiotic bacteria should be characterized when developing clinical applications for extended target populations (Isolauri et al., 2001).
2.1 General immunological aspects vis a vis immunomodulation and probiotics:

2.1.1 Control of antigen absorption in the gut:

Development of tolerance to probiotic antigens on the line of food antigens is an important requirement to pave way for the probiotics being counted as GRAS microbes.

The exposure of dynamic antigen load allows a myriad of antigens to be encountered by the small intestine through the passage of the enteric route. Immune exclusion by functional mucosal barrier of the gut removes most of the antigens (Sanderson et al., 1993). Lysosomal degradation is the second line of defence wherein transcytosis across the villous epithelium makes way for the elimination in the lysosomes of the antigen that have penetrated the mucosa. Unprocessed antigens are allowed to be transported via a minor pathway through M cells in the Peyer's patches (Ducroc et al., 1993; Isolauri et al., 1993). Antigens are presented to subjacent T cells; these differentiate into various effector cells that mediate active immune suppression and promote the differentiation of IgA-secreting B cells (Strober et al., 1998). Dietary antigens are converted to a tolerogenic form due to the absorption process across the intestinal mucosa. As a result of this hyporesponsiveness to antigens, for example, food proteins, oral tolerance is a hallmark of the intestinal immune system.

Antigens encountered through the enteric route face an immunologic hyporesponsiveness which is referred as oral tolerance (Strober et al., 1998; Strobel et al.,
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1998). Experimental animal studies have shown that the dose and frequency of a fed antigen impacts the sequence of tolerance acquisition. Clonal deletion or anergy is produced due to feeding of high doses of an antigen, while low doses of an antigen have been dealt with active suppression subsequent to the initiation of regulatory T cells in Peyer's patches. The regulatory T lymphocytes function through the production of suppressive cytokines, including interleukin 4, interleukin 10, and transforming growth factor β. Clonal deletion or anergy is preceded by the local production of interleukin 12, interferon γ (with consequent suppression of interleukin 4 and transforming growth factor β generation), and includes the apoptosis of T helper 1 cells. Local cytokine regulation, particularly transforming growth factor β-associated low-dose tolerance is proposed to be one among the major mechanisms by which the gut-associated lymphoid tissue maintains homeostasis (Strober et al., 1998; Isolauri et al., 2001).

All types of intraluminal antigens do not induce oral tolerance. Bacterial antigens in the Intraluminal area stimulate specific responses in the gut-associated lymphoid tissue. This may be described by the capacity of intraluminal bacterial antigens to bind to the epithelial cells, which lets antigen access via enterocytes and escapes tolerance generation in Peyer's patches. Such boost in immune responses in the gut-associated lymphoid tissue may give way for control of the metabolic activity and balance of the gut microflora (Strober et al., 1998). Epithelial cell adhesion capacities of antigens have been reported to be highly divergent and available probiotics have been categorized according to this property (Tuomola et al., 1998). Strong adhesion of antigens to epithelial cells is related with improved gut immune response. On the other hand, Duchmann et al (Duchmann et al., 1995) reported that...
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Healthy individuals are tolerant to their own microflora and that such tolerance is abrogated in patients with inflammatory bowel disease. Variation of the properties of the indigenous microflora by probiotic therapy upturned some immunologic disturbances typical of inflammatory bowel disease. These data advocate that candidate probiotic bacteria play an inconsistent role in immune regulation: improvement of gut-immune response and upgrading of oral tolerance. Such inconsistent regulation of the immune reaction to enteral antigens appears to be a continuous finding in the gut-related lymphoid tissue, and oral tolerance is reflected to be an associated effect of immune exclusion and suppression of systemic immune reaction, probably ascribed to the dual influence of the suppressor cytokine transforming growth factor β (Malin et al., 1996; Isolauri et al., 2001).

In the perspective of inflammation, the changed rate, route, and mode of antigen presentation may result in the abrogation of oral tolerance. Inflammation in the intestinal mucosa induced by viruses may be a reason of secondarily increased intestinal permeability to bacteria, or dietary antigens. A large amount of antigens may thus traverse the mucosal barrier, and the routes of transport may be changed. During the consequent mucosal dysfunction caused by immaturity, infection, or hypersensitivity reaction, the normal pattern of antigen handling is compromised, which may arouse anomalous immune responses and lead to sensitization. These data imply that allergic reaction to dietary antigens is caused by failure of the gut-associated lymphoid tissue to attain or preserve oral tolerance to these antigens (Sanderson et al., 1993; Isalauri et al., 1993; Fargeas et al., 1995).
2.1.2 Effect on the gut defense mechanisms due to intestinal flora:

Microbial colonization begins after birth, but the development of the intestinal microflora and the gut barrier is a gradual process. The maternal intestinal flora is a source of bacteria colonizing the newborn's intestine. Colonization is also determined by contact with surroundings. Initially, facultative anaerobic strains dominate. Thereafter, differences exist in the composition of species, mainly because of the type of diet. Breast-feeding encourages the growth of beneficial bacteria, whereas formula-fed infants have a more complex microflora made up of *bifidobacteria*, *bacteroides*, *clostridia*, and *streptococci*. After weaning, the composition of the microflora resembles that of the adult flora. Although bacteria are distributed throughout the intestine, the major concentration of microbes can be found in the large intestine (Salminen et al., 1998).

The adult human gut includes bacteria of transient and indigenous types. The mouth provides home to a complex micro-flora comprising of facultative and strict anaerobes, which embraces *streptococci*, *bacteroides*, *Lactobacilli*, and *yeasts*. The upper bowel (stomach, duodenum, and jejunum) has a sparse microflora with $\leq 1 \times 10^8$ colony-forming units/L contents. From the ileum and through the remainder of the digestive tract, bacterial concentrations gradually increase, reaching $1 \times 10^{11}$–$10^{12}$ colony-forming units/g in the colon. Up to 500 species of bacteria may be present in the adult human large intestine. Information from several reports indicated that 5 genera account for most of the viable forms of anaerobic bacteria in the large intestine: *Bacteroides*, *Eubacterium*, *Bifidobacterium*, *Peptostreptococcus*, and *Fusobacterium*. Various facultative and aerobic organisms are present in the colon.
Cumatively, it is projected that bacteria account for 35–50% of the volume of the contents in the human colon (Simon and Gorbach, 1986; Salminen et al., 1998).

An important constituent in the intestine's defense barrier is the gut microflora, as shown by amplified antigen transport across the gut mucosa in the absence of an intestinal microflora. This notion is further reinforced by a demonstration that the gut microflora prompt specific immune responses at a local and a systemic level. The gut flora has moreover been shown to induce and maintain oral tolerance in experimental animal models. The intestinal flora consents for perseverance of systemic hypo-responsiveness to an antigen and curtails the abrogation of hypo-responsiveness mediated by the *Escherichia coli* toxin (Kaila et al., 1992; Sutas et al., 1996; Salminen et al., 1998).

In addition to participation in tolerance induction, intestinal colonization acts as an important antigenic stimulus for the maturation of the gut-associated lymphoid tissue. The capability to generate IgA-producing cells progressively surges in response to intestinal antigenic stimulation, particularly the establishment of the gut microflora. Upon colonization, organisms have been reported to translocate to the mesenteric lymph node, but the number of translocating bacteria begins to shrink with the onset of specific IgA reaction, reflecting maturation of the intestine's immunologic defense mechanisms (Moreau et al., 1978; Shroff et al., 1995; Helgeland et al., 1996).
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The role of the intestinal microflora in oral tolerance induction to the IgE response was investigated in germfree mice. In distinction with control mice, germ free animals preserved a T-helper-2-type immune response, e.g., production of IgE antibodies, to orally administered ovalbumin. Abrogation of oral tolerance was owing to the lack of intestinal flora. Aberrant IgE reaction by germfree mice could be amended by the reconstitution of such flora at the neonatal stage, but not by any reconstitution exerted at a later age. These results advocate that in affecting the development of gut-associated lymphoid tissue at the neonatal stage, the intestinal bacterial flora plays central role in generating a T-helper-2 population that is vulnerable to oral tolerance induction (Sudo et al., 1997).

Study of the microflora expansion in vaginally born and caesarean delivery infants whose mothers had received prophylactic antibiotics, have shown to exhibit major differences in the culturable microflora (Gronlund et al., 2000). Differences were still observed at 6 months of age when a substantial proportion of children born by caesarean delivery were not colonized with Bacteroides fragilis. Colonization appeared to be associated with the maturation of humoral immune mechanisms. Fascinatingly, B. fragilis and, to a lesser extent, bifidobacteria, were significant in this respect because infants harbouring these organisms had more circulating IgA- and IgM-secreting cells. These results suggest that intestinal microflora is essential in human individuals and that qualitative differences in the composition of the microflora might affect immunologic homeostasis. The effect of gut microflora on the maturation of the gut immune defense culminates in early infancy when the mode
of immune responsiveness to antigens is consolidating (Holt et al., 1990; Isolauri et al., 1997).

2.1.3 Probiotics: a bacteriotherapy:

The demonstration that the gut microflora is an important constituent in the intestine's mucosal barrier has introduced the concept of probiotic therapy: therapeutic application of potentially beneficial microorganisms, which act as probiotics. A probiotic has been defined as a live microbial feed supplement that beneficially affects the host by improving its intestinal microbial balance. The definition is unsatisfactory for the purposes of human nutrition. Therefore, a European Commission concerted action program, coordinated by the International Life Sciences Institute, redefined probiotics as “A live microbial food ingredient that is beneficial to health”. A research group also demonstrated through their study a rational approach to harness the therapeutic potential of health-associated microbial communities to treat *C. difficile* disease and potentially other forms of intestinal dysbiosis, thereby using bacteriotherapy for treatment (Fuller et al., 1991; Salminen et al., 1998, Lawley et al., 2012).

The criteria for a microorganism to be defined as probiotic include that the strain be of human origin, are safe for human use, be stable in acid and bile, and adhere to the intestinal mucosa. Indian Council of Medical Research (ICMR) along with the Department of Biotechnology (DBT) defined a set of parameters required for a product/strain to be termed as ‘probiotic’. These include identification of the strain, *in vitro* screening for probiotic characteristics, animal studies to establish safety and *in vivo* animal and human studies to establish efficacy as well as many
other details. The genera most frequently used as probiotics are *Lactobacillus* and *Bifidobacterium* (Ouwehand et al., 1998, Ganguly et al., 2011).

### 2.1.4 A short review of the T helper system of immunity

Probiotics tends to act through means and modalities of the immune system that itself is so diverse and complex that various studies reporting differently and even in a contradictory fashion has to be taken notice thereof. As a result a review of the T helper system of cells becomes mandatory to understand the underlying means of immune system being used by probiotic microbes to alleviate the symptoms of disease.

The T helper cells (Th cells) are a kind of T cell that serves to play an important role in the immune system, particularly in the adaptive immune system. They orchestrate the activity of other immune cells by releasing T cell cytokines. They are crucial in B cell antibody class switching, in the activation and development of cytotoxic T cells, and in making best use of bactericidal activity of phagocytes such as macrophages.

Upon the expression of the surface protein CD4 the mature Th cells are referred to as CD4+ T cells. CD4+ T cells are usually regarded as having a pre-ordained role as helper T cells within the immune system. For instance, when an antigen presenting cell expresses an antigen on MHC class II, a CD4+ cell will help those cells via a combination of cell to cell interactions (e.g. CD40 and CD40L) and through cytokines. However, there are infrequent exceptions; for example, sub-groups of regulatory T cells, natural killer T cells, and cytotoxic T cells express CD4 (although cytotoxic examples have been witnessed in extremely low numbers in specific
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disease situations, they are commonly considered non-existent). All of the latter CD4+ T cell groups are not included as T helper cells (Alberts et al, 2002; Kuby et al, 2007).

**Activation of naïve helper T cells**

Naïve T cells after the succeeding T cell development become matured and leave the thymus and start to spread throughout the body, together with the lymph nodes. (Naïve T cells are those T cells that have never been introduced to the antigen that they are planned to respond to). The T cell receptor-CD3 complex is expressed by them like all the other T cells. The T cell receptor (TCR) comprises of both constant and variable regions. The variable region controls what antigen the T cell can respond to. CD4+ T cells have TCRs with an affinity for Class II MHC, and CD4 is tangled in determining MHC affinity all through maturation in the thymus. Class II MHC proteins are usually only found on the surface of dedicated antigen-presenting cells (APCs). Specialised antigen presenting cells are principally dendritic cells, macrophages and B cells, although B cells are the only cell group that expresses MHC Class II constitutively. Some APCs, such as follicular dendritic cells, also attach native (or unprocessed) antigens to their surface, but unprocessed antigens do not interact with T cells and are not involved in their activation. The antigens that bind to MHC proteins are always short peptides, 8-10 amino acids long for MHC Class I, and up to 25 or so for MHC Class II. (Alberts et al, 2002; Kuby et al, 2007).

**Signal 1 or Recognition of antigen by Th cell**
Foreign material (typically bacteria or viruses) which are endocytosed by professional antigen-presenting cells (APCs) during an immune response undergo processing and then travel from the site of infection to the lymph nodes. Once at the lymph nodes, the APC begin to present antigen peptides that are attached to Class II MHC, allowing CD4+ T cells that express the specific TCRs against the peptide/MHC complex to activate. The encounter and recognition of the antigen on an APC by a Th cell is followed by the TCR-CD3 complex binding strongly to the peptide-MHC complex being expressed on the surface of the professional APCs. A different section of the MHC molecule also binds to the CD4, a co-receptor of the TCR complex. These interactions bring these proteins closer together, allowing the intracellular kinases present on the TCR, CD3 and CD4 proteins to trigger each other via phosphorylation. With the help of a phosphatase present on the intracellular section of CD45 (common leukocyte antigen), these molecules stimulate major Th cell intracellular pathways. It is the first and primary pro-activation signal in a Th cell and hence these active pathways are referred to as Signal 1 of T cell activation. The same TCR pathways are employed for the reactivation of memory T cells upon subsequent encounters with a given antigen (Alberts et al, 2002; Kuby et al, 2007).

The APC and the Th cell adherence during Th cell activation may be assisted by the binding of the antigen-MHC to the TCR complex and CD4, but the primary molecules of adhesion in this cell interaction includes integrin protein LFA-1 on the T cell and ICAM on the APC. The role of the relatively bulky extracellular region of CD45 during cell interactions is rather unknown, but there are various isoforms of CD45 that change in size depending on the Th cell's activation and maturation status.
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For instance, CD45 shortens in length following Th activation (CD45RA+ to CD45RO+), but whether this alteration in length effects activation is unknown. It has been suggested that the larger CD45RA+ may reduce the accessibility of the T cell receptor for the antigen-MHC molecule, thereby necessitating an increase in the affinity (and specificity) of the T cell for activation. Once the activation has occurred however, CD45 shortens, allowing easier interactions and activation as an effector T helper cell (Alberts et al., 2002; Kuby et al., 2007).

Signal 2 or Verification of antigen by Th cell

The naïve T cell must activate a second independent biochemical pathway, known as Signal 2 after having received the first TCR/CD3 signal. This verification or substantiation step is a protective measure to ensure that a T cell is responding to a foreign antigen. The T cell assumes that it is auto-reactive if this second signal is not present during initial antigen exposure. This results in the cell becoming anergic (anergy is generated from the unprotected biochemical changes of Signal 1). Anergic cells will not respond to any antigen in the future, even if both signals are present later on. These cells are usually thought to circulate throughout the body with no value until they undergo apoptosis. The second signal comprises of an interaction between CD28 on the CD4+ T cell and the proteins CD80 (B7.1) or CD86 (B7.2) on the professional APCs. Both CD80 and CD86 stimulate the CD28 receptor. These proteins are also identified as co-stimulatory molecules. The importance of this verification stage, though essential for the activation of naïve helper T cells, is best revealed during the similar activation mechanism of CD8+ cytotoxic T cells. As naïve CD8+ T cells have no true bias towards foreign sources, these T cells must trust on the activation of CD28 for confirmation that they distinguish a foreign...
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antigen (as CD80/CD86 is only expressed by active APC's). CD28 plays a significant role in diminishing the threat of T cell auto-immunity against host antigens. Once the naïve T cell has both pathways activated, the biochemical changes encouraged by Signal 1 are altered, permitting the cell to activate instead of anergise. The second signal is then obsolete; only the first signal is essential for future activation. This is also true for memory T cells, which is one example of learned immunity. Faster responses follow upon reinfection because memory T cells have already undergone confirmation and can produce effector cells much faster (Alberts et al., 2002; Kuby et al., 2007; Peter et al. 1999).

Proliferation

After the completion of the two-signal activation, the T helper cell (Th) then permits itself to proliferate. It realizes this by releasing a powerful T cell growth factor called interleukin 2 (IL-2) which acts upon itself in an autocrine fashion. The alpha sub-unit of the IL-2 receptor (CD25 or IL-2R) is also produced by the activated T cells enabling a fully functional receptor that can bind with IL-2 and which in turn triggers the T cell's proliferation pathways. The autocrine or paracrine secretion of IL-2 can bind that same Th cell or neighbouring Th's via the IL-2R thereby leading the proliferation and clonal expansion. The Th cells receiving both signals of activation will then convert to Th0 cells (T helper 0) cell that secrete IL-2, IL-4 and interferon gamma (IFN-γ). Th1 or Th2 cells will then differentiate from the Th0 cells depending on cytokine environment. Th1 cell production is driven by IFN-γ while IL-10 and IL-4 inhibit Th1 cell production. Conversely, IL-4 drives Th2 cell production and IFN-γ inhibits Th2 cells. It should be noted that these cytokines are
pleiotropic and carry out many other functions of the immune response (Alberts et al., 2002; Kuby et al., 2007).

**Maturation**

The Th cell's progenitors segregate into effector Th cells, memory Th cells, and regulatory Th cells consequent to many cell generations. Effector Th cells secrete cytokines, proteins or peptides that rouse or interact with other leukocytes, including Th cells. Memory Th cells preserve the antigen affinity of the initially activated T cell, and are used to act as later effector cells during a second immune response (e.g. if there is re-infection of the host at a later stage). Regulatory T cells tend to reduce instead of encouraging immune function. Notwithstanding their low numbers during an infection, these cells are believed to play a significant role in the self-limitation of the immune system; they have been shown to avert the development of various autoimmune diseases (Alberts et al., 2002; Kuby et al., 2007; Peter et al., 1999).

**Determination of the effector T cell response**

A variety of immune cells are proficiently influenced by the Helper T cells, and the T cell response generated (including the extracellular signals such as cytokines) can be vital for a successful outcome from infection. In order to be effective, helper T cells must determine which cytokines will allow the immune system to be most useful or beneficial for the host. Considering exactly how helper T cells respond to immune challenges is currently of major interest in immunology, because such knowledge may be very useful in the management of disease and in increasing the effectiveness of vaccination (Alberts et al., 2002; Kuby et al, 2007).
Th1/Th2 Model for helper T cells

Proliferating helper T cells that progress into effector T cells distinguish into two major subtypes of cells known as Th1 and Th2 cells (also known as Type 1 and Type 2 helper T cells, respectively). The role of the host immunity effectors against intracellular bacteria and protozoa is played by the Th1 helper cells. They are activated by IL-12, IL-2 and their effector cytokine is IFN-γ. Macrophages as well as CD8 T cells, IgG B cells, and IFN-γ CD4 T cells are the main effector cells of Th1 immunity. The key Th1 transcription factors are STAT4 and T-bet. IFN-γ secreted by CD4 T cells can trigger macrophages to phagocytose and digest intracellular bacteria and protozoa. In addition, IFN-γ can activate iNOS to produce NOx free radicals to directly kill intracellular bacteria and protozoa. Th1 over stimulation against autoantigens will lead to Type 4 delayed-type hypersensitivity. Tuberculin reaction or Type 1 diabetes belong to this category of autoimmunity (Alberts et al, 2002; Kuby et al, 2007).

Th2 helper cell plays the host immunity effectors against multicellular helminths. They are activated by IL-4 and their effector cytokines are IL-4, IL-5, and IL-13. Eosinophils, basophils, and mast cells as well as IgE B cells, and IL-4/IL-5 CD4 T cells are the main effector cells. The main Th2 transcription factors are STAT6 and GATAs. IL-5 from CD4 T cells will stimulate eosinophils to attack helminths. Besides, IL-4 and IgE will rouse mast cells to release histamine, serotonin, and leukotriene to cause broncho-constriction, intestinal peristalsis, gastric fluid acidification to expel helminths. Th2 overactivation against autoantigen will cause...
Type I IgE-mediated allergy and hypersensitivity. Allergic rhinitis, atopic dermatitis, and asthma belong to this category of autoimmunity (Mosmann et al., 1986).

Although we know much about the various types of cytokine configurations helper T cells tend to produce, we have been able to understand less about how the patterns themselves are defined. Various evidences recommend that the kind of APC presenting the antigen to the T cell has foremost effect on its profile. Other indication suggests that the concentration of antigen presented to the T cell during primary activation influences its choice. The occurrence of some cytokines (such as the ones mentioned above) will also impact the response that will finally be generated, but our understanding is rather incomplete.

**Th17 helper cells**

Th17 helper cells facilitate host immunity against the extracellular bacteria and fungi. This effector cell subtype is activated by IL-6 and TGF beta. Its main effector cytokines are IL-17a, IL-21, and IL-22. The main Th17 effector cells are neutrophils as well as IgM/IgA B cells, and IL-17 CD4 T cells. The key Th17 transcription factors are STAT3 and RORgamma. TNF alpha can stimulate neutrophils to kill extracellular bacteria and fungi. Besides, IL-6 can increase expression of the complement system to directly kill extracellular bacteria and fungi. Th17 overactivation against autoantigen will cause type 3 immune complex and complement-mediated hypersensitivity. Rheumatoid arthritis or Arthus reaction belong to this category (Harrington et al., 2005).
Bone erosion caused by mature osteoclast cells is typical in patients with rheumatoid arthritis. Activated T helper cells such as Th1, Th2, and Th17 are found in the synovial cavity during the time of inflammation due to rheumatoid arthritis. The known mechanisms associated with the differentiation of osteoclast precursors into mature osteoclasts include the signaling molecules produced by immune-associate cells, as well as the direct cell to cell contact of osteoblasts and osteoclast precursors. Nevertheless, it has been proposed that Th17 can also play a more major role in osteoclast differentiation via cell to cell contact with osteoclast precursors (Fumoto et al., 2013; Won et al., 2011).

**THαβ helper cells**

The host immunity against viruses is provided by THαβ helper cells. Their differentiation is triggered by IFN alpha/beta or IL-10. Their main effector cytokine is IL-10. Their main effector cells are NK cells as well as CD8 T cells, IgG B cells, and IL-10 CD4 T cells. The key THαβ transcription factors are STAT1 and STAT3 as well as IRFs. IL-10 from CD4 T cells triggers NK cells' ADCC to apoptose virus-infected cells and to prompt host as well as viral DNA fragmentation. IFN alpha/beta can subdue transcription to avoid virus replication and transmission. Overactivation of THαβ against autoantigen will lead to type 2 antibody-dependent cytotoxic hypersensitivity. Myasthenia gravis or Graves' disease belong to this category (Hu, Wanchung, 2007).

**Confines to the Th1/Th2 model**
The communications between cytokines from the Th1/Th2 model can be more intricate in some animals. For example, the Th2 cytokine IL-10 inhibits cytokine production of both Th subsets in humans. Human IL-10 (hIL-10) suppresses the proliferation and cytokine production of all T cells and the activity of macrophages, but carries on to stimulate the plasma cells, ensuring that the antibody manufacturing still occurs. As such, hIL-10 is not assumed to truly promote the Th2 response in humans, but acts to avert over-stimulation of helper T cells while still maximising the production of antibodies. Other varieties of T cells also exist that can affect the expression and activation of helper T cells, such as natural regulatory T cells, along with less common cytokine profiles such as the Th3 subset of helper T cells. Terms such as "regulatory" and "suppression" have become ambiguous after the discovery that helper CD4+ T cells are also accomplished of regulating (and suppressing) their own responses separate from the dedicated regulatory T cells (Fumoto et al., 2013; Won et al., 2011).

A major difference between regulatory T cells and effector T cells is that regulatory T cells characteristically serve to regulate and deactivate the immune response, while effector T cell groups generally begin with immune-promoting cytokines and then switch to inhibitory cytokines later in their life cycle. The second one is a feature of Th3 cells, which transform into a regulatory subset after its initial activation and cytokine production. Both regulatory T cells and Th3 cells generate the cytokine transforming growth factor-beta (TGF-β) and IL-10. Both cytokines are inhibitory to helper T cells; TGF-β subdues the activity of most of the immune system. Evidences tend to suggest that TGF-β may not suppress activated Th2 cells as efficiently as it
might suppress naive cells, but it is not typically considered a Th2 cytokine (Kidd P., 2003; Fumoto et al., 2013).

The description of another unique T helper subtype, T helper 17 cells (Th17) has cast additional doubt on the basic Th1/Th2 model. These IL-17 producing cells were primarily described as a pathogenic population implicated in autoimmunity but are now thought to have their own distinct effector and regulatory functions. Of note, recent evidence advocates that functional plasticity is an intrinsic capacity of T helper cells. Indeed, a study in mice demonstrated that Th17 cells transform into Th1 cells in vivo (Hirota et al., 2011). A follow up study furthermore showed that extensive T helper cell plasticity is also noticeable in man (Larsen et al., 2011).

Many of the cytokines are also expressed by other immune cells, and it is becoming clear that while the original Th1/Th2 model is illuminating and gives insight into the functions of helper T cells, it is far too simple to define its entire role or actions. Some immunologists question the model completely, as some in vivo studies suggest that individual helper T cells usually do not match the precise cytokine outlines of the Th model, and many cells express cytokines from both profiles. Nevertheless, the Th model has still played an important role in evolving our understanding of the roles and behaviour of helper T cells and the cytokines they produce in the course of an immune response (Nakayamada et al., 2012).

Stockinger et al. have reported the existence of another T helper subset. Th9 cells are claimed to be an IL9 (interleukin 9) – producing T cell subset dedicated on defending helminth infections (Veldhoen et al., 2008).
2.2 Growth in varying pH and *Lactobacilli*:

After ingestion, probiotics have to survive the adverse gastric environment in order to reach the intestine and exert their beneficial effects. Several studies have been conducted using simulated gastric juices in order to select acid tolerant strains and understand the mechanisms behind their acid tolerance (Charteri *et al.*, 1998; Corcoran *et al.*, 2005; Maragkoudakis *et al.*, 2006). In order to improve the survival of strains the research has mainly focused either on strategies to adapt the cells to low pH (de Angelis *et al.*, 2001) or on the potentially protective effects of the food matrix or food ingredients (Charalampopoulos *et al.*, 2003; Desmond *et al.*, 2002; Tarahomjoo *et al.*, 2008).

*Lactobacillus* species are considered intrinsically resistant to acid (Tannock, 2004). *Lactobacillus* species as probiotics must survive in the acidic gastric environment if they are to reach the small intestine and colonize the host, thereby imparting their benefits. Although there are differences between species and strains, organisms generally exhibit increased sensitivity at pH values below 3.0 (Jin *et al.*, 1998; Ronka *et al.*, 2003).

Hence, acid tolerance is accepted as one of the desirable properties used to select potentially probiotic strains.

Most of the probiotic *Lactobacilli* in human foods are supplied in highly concentrated forms containing more than $10^{10}$ cfu/g$^3$. It is recommended that probiotic bacteria are delivered in high numbers in food products (at least $10^7$ c.f.u.)
ml⁻¹ or g⁻¹) for efficacy (Ishibashi et al., 1993), although this number depends on a number of factors, such as the particular strain used, the food processing conditions and gastric transit survival. In this regard, acid tolerance is an important probiotic trait for survival during gastric transit and in fermented food products (Holzapfel et al., 2002). A number of approaches have been investigated for enhancing survival of probiotic bacteria in acid conditions, including physical protection via the choice of food system (Conway et al., 1987; Gardiner et al., 1999), encapsulation (Desmond et al., 2002; O’Riordan et al., 2001) and incorporation of fermentable substrates in the acid environment (Charalampopoulos et al., 2003; Corcoran et al., 2005). Lb. rhamnosus GG has been shown to survive passage through the adult human gastrointestinal tract (Saxelin et al., 1995) and could survive in conditions as low as pH 2.5 (Jacobsen et al., 1999).

In addition, the exploitation of rich media, such as acidified MRS medium, may provide protection to bacteria by providing energy and metabolic precursors (Corcoran et al., 2005).

*L. rhamnosus* GG survival in acidic conditions occurred only in the presence of sugars that it could metabolize efficiently. To confirm the involvement of glycolysis in the glucose effect, iodoacetic acid was used to inhibit glyceraldehyde-3-phosphate dehydrogenase (GAPDH) activity. The reduction in GAPDH activity caused survival to decrease by 8.30 log₁₀ CFU ml⁻¹ in the presence of glucose. The data indicate that glucose provides ATP to F0F1-ATPase via glycolysis, enabling proton exclusion and thereby enhancing survival during gastric transit (Corcoran et al., 2005).
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$F_0F_1$-ATPase requires ATP for expulsion of $H^+$ from the cell, thereby maintaining pH homeostasis and cell viability. The accumulation of ATP is as a result of energy-generating factories, such as the glycolytic system. Studies found that *Lactobacilli* could sequester glucose to survive in a simulated gastric environment. It was also found that the inhibition of glycolysis affected the ability of *L. rhamnosus* GG to survive in simulated gastric juice in the presence of 19.4 mM glucose. Glucose in acid conditions can therefore enhance probiotic survival by providing the ATP pool required, permitting optimal $H^+$ extrusion by $F_0F_1$-ATPase. Such a mechanism can provide more effective delivery of viable probiotic *Lactobacilli* to the human GIT (Corcoran *et al.*, 2005).

It is now apparent that a combination of constitutive and inducible strategies which result in the removal of protons ($H^+$), alkanization of the external environment, changes in the composition of the cell envelope, production of general shock proteins and chaperones, expression of transcriptional regulators, and responses to changes in cell density can all contribute to survival. These mechanisms counter the negative impact of a reduction in cytoplasmic pH, which can include loss of activity of the relatively acid-sensitive glycolytic enzymes (which severely affects the ability to produce ATP) and structural damage to the cell membrane and macromolecules such as DNA and proteins (Paul *et al.*, 2003).
The exposure to shifting pH encountered in the stomach due to the gastric acid negatively affects the proton motive force (PMF) across the membrane as a result of the accumulation of protons inside the cell (Corcoran et al., 2008). Stress of the acid causes damage to DNA and proteins in addition to the cell membrane structural damage (van de Guchte et al., 2002). Bacterial cells are naturally fortified with a surplus of defense mechanisms to augment survival in hostile pH environments. These include proton pumps, decarboxylases and transporters to combat decreases in intracellular pH (Corcoran et al., 2008; De Angelis et al., 2004; Sugimoto et al., 2008; van Schaik et al., 2005).

The putative polyphosphate kinase gene responsible for poly P synthesis of Bifidobacterium scardovii has been recently connected to the oxidative stress response as well as providing protection against other environmental stresses such as low pH (Qian et al., 2011). Lactobacillus casei ATCC 334 showed highest survival to acid stress when exposed to pH 4.5 for 10 or 20 minutes (Broadbent et al., 2010). Whole genome DNA microarrays revealed 104 genes getting up-regulated and 216 genes showed down-regulation after 20 minutes at pH 4.5. Histidine accumulation and malolactic fermentation were also revealed as important features of acid adaptation in Lactobacillus casei. Malolactic enzyme was found up-regulated 16-fold and 7 fold following exposure of 5 and 20 minutes to acid, respectively. This enzyme functions to decarboxylate L-malate to L-lactate and CO₂, thus contributing to alkalisation of the cytoplasm and allowing the production of ATP through H⁺-ATPase (Poolman et al., 1991; Renault et al., 1988).
Another study revealed the up-regulation of cluster of eight genes involved in histidine biosynthesis when the transcriptome of cells was exposed to acid for 20 minutes. The authors postulated that histidine accumulation may contribute to the buffering capacity within the cell and that histidine accumulation may enhance acid resistance in bacteria. Addition of either 30 mM histidine or 30 mM malate during acid exposure (pH 2.5) for 60 minutes or 2 hours interestingly improved cell survival more than 100-fold or more than $10^7$-fold, respectively. A role for the luxS gene in the acid stress response of *Lactobacilli* was suggested with a significantly increased activity of the LuxS-mediated quorum sensing system, which is responsible for generating the universal signaling molecule called autoinducer-2 (AI-2), following exposure to acid shock in *Lactobacillus acidophilus* NCFM and *Lactobacillus rhamnosus* GG suggesting. Usp1, the gene encoding the universal stress protein has also been suggested to be a key mediator in the acid stress response of *Lactobacillus plantarum*. Studies in *Escherichia coli* have indeed demonstrated that Usp1 from *Lactobacillus-plantarum* inactivates the negative regulator PadR involved in the phenolic acid stress response by negatively regulating padA, a gene which encodes the phenolic acid decarboxylase enzyme (Moslehi et al., 2009; Gury et al., 2009).

Highest survival was reported at pH 4.5 *Lactobacillus casei* ATCC 334 was exposed to a broad range of acid stresses for 10 or 20 minutes while trying to improve the acid tolerance of the strain (Broadbent et al., 2010). A dramatic increase in the number of responsive genes following the 20 minute treatment (320 genes with altered expression levels) was observed when an assessment was made of the transcriptional responses of the strain ensuing 5 and 20 minutes of exposure to this
pH level. A trend of down regulation was recorded in the majority of genes. This was mainly obvious for genes involved in information storage and processing including translation, ribosomal structure and biogenesis, transcription, DNA replication, recombination and repair as well as genes involved in cellular processes such as protein turnover and remarkably stress response genes and those involved in cell secretion and cell envelope biogenesis (Mills et al., 2011).

Malolactic enzyme and genes involved in amino acid transport and metabolisms, including those involved in the transport of histidine were found to be up-regulated. Another unique observation reported in this study following acid adaptation for 20 minutes was the up-regulation of genes involved in the mobile DNA elements category. Three genes associated with phospholipid turnover: an acetyl transferase, an esterase, and a putative membrane-associated phospholipid phosphatase were observed to show a rather poor up-regulation following acid exposure compared to the other up-regulated genes (Mills et al., 2011).

Saturated and cyclopropane fatty acids in the cytoplasmic membrane were nonetheless found at a higher level than control cells which may be associated to the up-regulation (Though comparatively less up-regulated) of the three phospholipid-associated genes. Studies from other groups have also demonstrated that acid stress makes changes in the cytoplasmic membrane fatty acid composition in Lactobacillus (Montanari et al., 2010; Streit et al., 2008).
2.3 Growth in bile and *Lactobacilli*:

Indigenous microbiota of the intestinal tract is exposed to bile acids, which are products of cholesterol metabolism in the liver and play an important role in the digestive process due to their amphipathic nature. When selecting lactic acid bacteria for use as dietary adjuncts, a number of factors should be considered. While the functionality of probiotics depends on their ability to survive and colonize the gastrointestinal tract, resistance of cells to bile acids is a strictly necessary property (Maria *et al.*, 2006). In order to reach the colon in a viable state, they must cope with specific stress challenges throughout the gastrointestinal tract, among which the presence of bile in the upper parts of the small intestine is one of the main ones. The main components of bile are bile acids, which are produced and conjugated with the amino acids glycine or taurine in the liver, to generate conjugated bile salts (Hofmann *et al.*, 1994).

Due to its lipophilic character, bacterial membranes represent one of the main targets of bile that disrupts the structure of bacterial envelopes, affecting both cell and colony morphology (Suskovic *et al.*, 2000; Margolles *et al.*, 2003; Kurdi *et al.*, 2006). This effect has been evaluated and used as a bile salt resistance marker in certain *Lactobacillus* strains, since rough colonies are more sensitive than smooth colonies, probably in connection to changes in envelope architecture (Suskovic *et al.*, 2000). Furthermore, changes in the lipid composition of bacterial membranes have been described following bile exposure in *Lactobacilli* (Gómez-Zavaglia *et al.*, 2002; Kociubinski *et al.*, 2002; Taranto *et al.*, 2003; Ruiz *et al.*, 2007; Ruiz *et al.*, 2013).
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Natural tolerance to bile salts was initially associated with the presence of bile salt hydrolase activity (De Smet et al., 1994; Moser et al., 2001); however, different research works showed that, at least in Lactobacilli, bile salt resistance could not be correlated with the presence of this enzyme (Gilliland et al., 1977; Moser et al., 2001; Schmidt et al., 2001). Furthermore, conjugated bile acids are less inhibitory than free bile acids (cholic and deoxycholic) toward intestinal aerobic and anaerobic bacteria (Floch et al., 1972). Significant variations in bile tolerance have been reported among Lactobacillus species and strains (Chateau et al., 1993; De Boever et al., 2000; Gilliland et., 1977). In Gram-positive bacteria, the toxicity pattern of bile acids resembles that of detergents such as SDS (Schmidt et al., 2001; Begley et al., 2002; Flahaut et al., 1996); however, the actual effects of bile acids on the bacterial cell, and consequently the mechanisms of tolerance/resistance, have not been clearly established. Through isolation of bile-salt-sensitive mutants in Enterococcus faecalis and Listeria monocytogenes, it was shown that survival in a bile-rich environment relies on multiple factors. Different loci related to this effect were identified, such as stress response systems and transcriptional regulators, elements involved in the maintenance of the cell envelope, energy metabolism, amino acid transport (putative role in pH homeostasis) and fatty acid or isoprenoid biosynthesis (Begley et al., 2002; Flahaut et al., 1996).

Bile is stored in the gall bladder and flows from there to the duodenum during digestion, facilitating the solubilization and absorption of dietary fats. Thus, under normal physiological conditions, our intestine holds a bile salt concentration gradient ranging from more than 40 mM to less than 1 mM – equivalent to a range between
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2% and 0.05% – which is responsible, among other factors, for shaping the microbial community profile found in our gut (Ruiz et al., 2013; Islam et al., 2011).

After ingestion, probiotics must be able to survive the detergent properties of bile acids in the small intestine. The concerted efforts of the potential probiotic candidate are to efflux out the bile as much as possible before it is overcome and the cell gets damaged beyond repair.

*L. monocytogenes* which had been isolated from the human gallbladder was found to have a noticeable ability to endure the stresses faced in the upper small intestine and had been linked to its ability to tolerate high bile concentrations (Briones et al., 2011; Hardy et al., 2004). A new bile resistance mechanism, termed BilE was identified in *L. monocytogenes* and was shown to operate by excluding bile from the cell (Mills et al., 2011; Sleator et al., 2005).

It has been reported that the heterologous expression of BilE in *L. lactis* NZ9800 and *B. breve* UCC2003 produced modified strains with enhanced capabilities to survive in the presence of bile (Watson et al., 2008). This study exemplified it with the observation that the *bilE*-containing strain of *L. lactis* exhibited a 2.5 log enhanced resistance (compared to control) following a 20 minute exposure to 1% porcine bile, and a similar result was also observed for the *bilE*-containing strain of *B. breve*. In addition, both genetically modified strains were shown to exhibit an enhanced ability to survive in vivo conditions using mouse models. This was particularly noteworthy for the *bilE*-containing *L. lactis* strain considering that it is generally not considered...
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to be a gut inhabitant due to its poor survival rates in the GIT. The BilE\(^+\) \textit{L. lactis} strain was indeed found to be present at high levels in murine faeces 3 days after the inoculation whereas the BilE\(^-\) \textit{L. lactis} strain could not be detected in the faecal samples on day 2 after inoculation. The murine GI tract was shown to be colonised by both BilE\(^+\) and BilE\(^-\) strains of \textit{B. breve} at relatively similar rates (10\(^7\) cfu/g of faeces), the persistence of the BilE\(^+\) strain had begun to differ considerably 12 days after inoculation. By day 19 the BilE\(^-\) strain had reached a level of 1 x 10\(^5\) cfu/g of faeces, whereas the BilE\(^+\) strain continued at a level of 4.5 x 10\(^7\) cfu/g of faeces. Moreover, by day 19 the levels of the BilE\(^+\) strain in the small intestine, caecum and large intestine were markedly higher compared to the control strain. This was a particularly important finding for the small intestine, a region of the GIT which has the highest levels of bile (Begley \textit{et al.}, 2005). Indeed, the persistence levels of the genetically modified strain were 100 fold greater than the persistence levels of the control. The BilE\(^+\) strain also enhanced clearance of \textit{L. monocytogenes} from the liver at significant levels when compared to the control strain (Mills \textit{et al.}, 2011).

DNA microarray of the whole genome was engaged to determine the transcriptional response of \textit{Lactobacillus reuteri} ATCC 55730 to bile. Differential expression of a wide variety of genes involved in cell envelope stress, protein denaturation and DNA damage was observed (Whitehead \textit{et al.}, 2009).

Mutating a Clp chaperone, a putative esterase and a gene of unknown function, decreased the survival of \textit{Lactobacillus reuteri} in the presence of bile whereas the capacity of the strain to restart growth in the presence of bile was hampered by
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mutating two operon genes, a multidrug resistance (MDR) transporter and a gene of unknown function, suggesting their importance in the bile stress response. The significance of MDR transporters for the bile stress response of *Lactobacillus acidophilus* was also revealed by Pfeiler and Klaenhammer (Pfeiler et al., 2009). Indeed, of the ten most highly induced genes in *Lactobacillus acidophilus* in the presence of bile, two were shown to encode MDR transporters. These transporters function by extruding structurally unrelated compounds from the cell including antibiotics and bile salts (Pfeiler et al., 2009). MDR transporters were also shown to be present in *B. longum* and *B. breve* following exposure to sub-inhibitory concentrations of bile. Bile resistance was conferred on the heterologous host *E. coli* due to expression of the MDR transporter from *B. longum* and it survived when exposed to 3% bile. But the genetically altered strain exhibited a reduced growth rate; thereby suggesting that the production of the MDR transporter was toxic for the cells (Gueimonde et al., 2009).

A gene responsible for S-layer production in *Lactobacillus acidophilus* ATCC 4356, slpA, was reported to be induced in bile concentrations ranging from 0.01-0.05% (Khaleghi et al., 2010). S-layer proteins are external bacterial structures which have been associated with protection against hostile environmental elements and the establishment of *Lactobacillus acidophilus* in the GIT. However, the expression of slpA in 0.1% bile was lower than that recorded in 0.02 and 0.05% bile although the level of Slayer protein on the cell surface improved in concentration (Avall et al., 2005; Frece et al., 2005; Kos et al., 2003).
Hence it was suggested that slpB, rather than slpA, may be expressed when conditions were unfavorable for growth (Khaleghi et al., 2010). A putative aggregation-promoting factor (Apf) of *Lactobacillus acidophilus* NCFM had been shown to be associated with the survival of the strain during passage through the GIT and may even be involved in bacterium-host interactions. Practically it was reported that a Δapf deletion mutant was much more susceptible to bile and detergent, and survival rates of the mutant strain were found to be reduced in simulated gastric juice compared to the control. It was shown to reduce the adherence of stationary phase mutant cells to an intestinal epithelial cell line. Overall, the results of the study suggest that Apf-like proteins are important for the gastrointestinal robustness of *Lactobacillus acidophilus* (Goh et al., 2010).

The up-regulation of a gene encoding cyclopropane-fatty-acyl-phospholipid synthase in *Lactobacillus rhamnosus* GG in response to bile stress was demonstrated by Koskenniemi's group, using both transcriptomics and strategic proteomics approaches, although the level of upregulation was not deemed to be statistically significant. This study also revealed the repression of EPS-encoding genes due to bile shock. It was postulated that EPS served to protect *Lactobacillus rhamnosus* GG cells from the punitive environmental conditions of the stomach (Koskenniemi et al., 2011). Nevertheless, the availability of bile serves as a signal of gut entrance and hence down-regulation in EPS production to allow better adherence of the bacterium to the intestinal cells. Expression of the genes involved in the D-alanylation of the negatively charged lipoteichoic acids were also reported to be upregulated in reaction to bile strain which was also seen for *Lactobacillus plantarum* (Bron et al., 2006).
These strategies serve to increase the positive surface charge possibly aiding to repulse the cationic compounds in bile. Indeed, change of surface charge has also been related with resistance to cationic peptides. Numerous two-component systems, multi-drug transporters, the $F_1F_0$-ATP synthase and a bile salt hydrolase were also up-regulated in reaction to bile stress as well as quite a few chaperones and proteases directly involved in the stress response (Kovacs et al., 2006; Peschel et al., 1999).

2.4 Effect of antibiotics and *Lactobacilli*:

In 1921, a Scottish bacteriologist, Alexander Fleming suggested the presence of a diffusible substance in the nasal secretion that affected the bacterial growth. The substance was a protein and Fleming named the substance as lysozyme, which was found to be a potent enzyme capable of dissolving the cell wall and causing lysis in many gram-positive (Steffee et al., 1992). The discovery of lysozyme paved the path for the later serendipitous discovery of penicillin.

The discovery of potent, chemically diverse, and relatively non-toxic antibiotics derived from environmental bacteria and fungi, paved the way for the ‘golden era’ of antibiotic discovery (1945–1960) during which most of the chemical classes of antibiotics now in clinical use were first characterized. This period was followed by the extensive medicinal chemical elaboration of these chemical scaffolds over the next decade (1970–1980) to modify these drugs to improve pharmacology and evade antibiotic resistance – the golden age of antibiotic medicinal chemistry (Wright, 2007).
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Once the antibiotic was used widely, resistant strains capable of inactivating the drug became prevalent. Drug-resistant strains initially appeared in hospitals, where utilization of antibiotics was greatest (Levy, 1998).

Interestingly, the identification of a bacterial penicillinase before the use of the antibiotic can now be appreciated in the light of recent findings that a large number of antibiotic resistance genes are components of natural microbial populations (D’Costa et al., 2006).

Heavy metals were used for disease treatment for centuries prior to the use of antibiotics and this may have selected for genes encoding both heavy metal and antibiotic resistance (Baker-Austin et al., 2006). Retrospective studies show that resistance genes were present in bacteria that did not produce antibiotics before the widespread dissemination of the drugs. Out of 30 E. coli strains that were lyophilized before 1950, 4 were resistant to the 8 tested antibiotics, and each resistance element could conjugate into E. coli (Smith, 1967). In another analysis of 433 enterobacterial strains collected from around the world between 1917 and 1952 (known as the Murray collection), 24% could transfer plasmids and 11 strains were resistant to ampicillin or tetracycline (Hughes et al., 1983). Thus, the determinants of antibiotic resistance existed naturally and were probably subjected to horizontal transfer long before the excessive selection pressure imposed in the antibiotic era.
Most of the strains of *Lactobacilli* had been found to be resistant to high concentrations of chloramphenicol, aztreonam, norfloxacin, ciprofloxacin, ceftazidime, ceftriaxone, and metronidazole. Susceptibility to other antibiotics (rifampicin, erytromicin, novobiocin, vancomycin, ampicillin, tetracycline, clarithromycin, imipenem, and cefotaxime) were shown to depend on each particular *Lactobacillus* strain. On the other hand, no correlation had been obtained with the disc diffusion method and the MICs results (Virginia *et al.*, 2006).

Resistance or susceptibility to vancomycin has deserved a special consideration in terms of classification of lactic acid bacteria, mainly for *Lactobacilli* related with human infections or isolated from food (Felten *et al.*, 1999; Kneifel *et al.*, 1994; Simpson *et al.*, 1988; Holliman *et al.*, 1988). Hamilton and Shah have used the susceptibility to vancomycin as an aid to identify *Lactobacillus* species (Hamilton *et al.*, 1998). Simpson and Felten *et al* have associated sensitivity to vancomycin with the *Lactobacillus* acidophilus group or those originally called “Thermobacteria” while Simpson has detected resistance to vancomycin in lactic acid bacteria belonging to the “Betabacteria” group (Simpson *et al.*, 1988; Felten *et al.*, 1999). However, Klein *et al.*, and Griffiths *et al.*, have reported resistance to vancomycin in different *L. acidophilus* strains isolated from clinical samples. According to the results obtained in this work, 4 of 6 *Lactobacilli* were able to grow at concentrations lower than 1 μg/mL of vancomycin. *L. crispatus* and *L. salivarius*, both homofermentatives (Thermobacteria), were able to grow at vancomycin concentrations higher than 10 and 1000 μg, respectively (Klein *et al.*, 1998; Griffiths *et al*; 1992).
The most frequently used antibiotics for the management of bacterial vaginosis are the metronidazole and clindamycin. Candidate probiotic *Lactobacillus* strains were able to grow at high concentrations of metronidazole and clindamycin, except for *L. acidophilus* CRL1251 and *L. salivarius* CRL1328 that did not grow at concentrations as low as 0.1 µg/mL of the last antibiotic. Such observations suggest that selected strains could be used for a restoration therapy together with the antimicrobial bacterial vaginosis treatment. Simoes *et al.* have also studied the effect of metronidazole on the growth of vaginal *Lactobacilli*. These authors have observed partial and complete inhibition at concentration above 1000 µg/mL while they have reported a stimulating effect at concentrations between 128 µg/mL and 256 µg/mL. Carlstedt-Duke *et al.* have detected a low effect of clindamycin on *Lactobacilli* when employing this antibiotic simultaneously with the lactic acid bacteria to restore the normal flora of the gut of rats (Simoes *et al.*, 2001; Carlstedt *et al.*, 1987).

Plasmids were not found in the strains taken for study and hence the extra chromosomal elements could not be associated with the antimicrobial resistance of candidate probiotic *Lactobacilli*. This observation would indicate a low probability of antibiotic resistance transmission to pathogenic microorganisms. However, other different methods should have been employed to confirm the absence of plasmids, mainly considering that *L. salivarius* CRL1328 is an aggregating strain able to produce bacteriocins, both characteristics frequently associated with extra chromosomal DNA (Reniero *et al*., 1992; Wang *et al*., 1997).
A lot of studies need to be undertaken to define the adequate and standardized method to study the antimicrobial susceptibility of the *Lactobacillus* genus. The use of LAPTg and MRS as base media for the disc diffusion method deserves further studies because the growth of Lactobacilli is more confluent on these rich media as compared to the generally used Muller Hinton media for testing antibiotic susceptibility. However, determination of the MICs is, up to date, the only reliable test to predict the susceptibility or the resistance to antibiotics of *Lactobacillus* strains (Virginia *et al.*, 2006).

There is a lack of agreement on the resistance/susceptibility breakpoints for most antimicrobials in LAB. Differentiating between intrinsic, nonspecific, and acquired resistance is difficult and requires that the antimicrobial-resistance patterns of many LAB species from different sources be compared. This is a very important task considering the fact that genes conferring resistance to several antimicrobials (i.e., chloramphenicol, erythromycin, streptomycin, tetracycline, and vancomycin) located on transferable genetic elements (plasmids or transposons) have already been characterized in lactococci, *Lactobacilli*, and enterococci from foods. Furthermore, under certain circumstances, LAB strains themselves have been reported to cause infections in humans (Ana *et al.*, 2005).

All analyzed LAB strains in a study were found resistant to metronidazole since LAB have no hydrogenase activity. All tested strains were shown to be susceptible to the lowest concentration of piperacillin and piperacillin plus tazobactam tested. The MICs of the remaining antibiotics showed a certain degree of variability. The group also found strains with clinical resistance for all antibiotics, except for amoxycillin.
and moxifloxacin, for which, breakpoints have yet not been determined. MICs for the amoxycillin–clavulanic acid mixture were always lower than those of amoxycillin alone, but followed the same pattern (Lennette et al., 1985; Church et al., 1996).

Cell wall synthesis inhibiting antibiotics did not show very high MICs. The resistance of LAB species to high levels of cefoxitin have been repeatedly observed. Cell-wall impermeability seems to be the main mechanism of resistance to inhibitors of cell-wall synthesis (penicillins and cephalosporins), since LAB species lack cytochrome-mediated electron transport. Nevertheless, the mutual aid of nonspecific mechanisms, such as multi-drug transporters and defective cell wall autolytic systems, may also account for the differences between strains (Ana et al., 2005; Putnam et al., 2001; Kim et al., 1982).

All Lactobacillus species were found to be resistant to high concentrations of vancomycin and this resistance of Lactobacillus spp. to vancomycin was suggested to be due to the presence of D-Ala-D-Lac as the normal dipeptide in their peptidoglycan (Klein et al. 2000). The MICs of antibiotics affecting the synthesis of proteins showed the greatest variation between species and strains with the exception of vancomycin. Most strains were clearly susceptible for all other antibiotics of this group (chloramphenicol, erythromycin, clindamycin, and tetracycline), although a few moderate to strongly resistant strains were also recorded. Some strains showed resistance to more than one of these antibiotics.
Even a few number of the normal starter LAB strains showing antibiotic resistance justifies performing antibiotic-susceptibility assays to avoid including antibiotic-resistant strains in starter cultures. Actually, several of the types and levels of resistance found are well-suited with transmissible genes. Compared with the results of surveys of strains from the pre-antibiotic era, in which no resistance was found at all, the present discoveries recommend that the antibiotic pressure on LAB from the wide use of antibiotics, in veterinary medicine and agriculture for instance, might be contributing to the dissemination of resistances into ecological niches of these GRAS bacterial group (Cogan 1972; Orberg and Sandine 1985; Katla et al. 200).

2.5 Antipathogenic character and Lactobacilli:

The interactions of lactic acid bacteria, particularly the Lactobacillus spp., with other bacteria have been widely researched in food products and especially in fermented foods (Ahn and Stiles, 1990; Kalyoussef et al., 2012). The ability to thrive and replace a variety of pathogenic and other microbes in the fermented food product with a variety of adverse conditions is an interesting aspect of the lactic acid bacteria (Sekwati-Monang et al., 2012). The antibacterial effect has been ascribed to the production of antibiotics or antibiotic-like substances, the effect of hydrogen peroxide production as well as the lactic acid produced as a result of fermentation (Hueh et al., 2012; Gupta et al., 1998; Cadieux et al., 2009, Atassi and Servin, 2010). The Lactobacillus isolates from honey have also been shown to have antibacterial activities against multiple antibiotic resistant pathogens though the activity varied with respect to different isolates from varying sources of honey (Aween et al., 2012).
The probiotic action of microorganisms against virulence factors from intestinal pathogens have also been studied and are gaining a lot of momentum now (Bolla et al., 2012). There is abundant evidence where specific strains selected from the healthy gut microflora exhibit powerful antipathogenic and anti-inflammatory capabilities, and are consequently involved with enhanced colonisation resistance in the intestine (Isolauri et al., 2002). Interactions of lactic acid bacteria and antibiotic resistant pathogens have been shown to have led to the elimination of 99% of the pathogenic cells within 24 h (Karska-Wysocki et al., 2010).

Urinary tract infections are common clinical entities occurring in a variety of pediatric patient groups and are frequently accompanied with urologic abnormalities. This can lead to end stage renal failure or hypertension if continued. *Escherichia coli* (*E. coli*), which are the predominant facultative anaerobes of the gastrointestinal tract, are the major cause of urinary tract infections (UTIs), the second most frequently isolated pathogen in neonatal sepsis, and the most common pathogen isolated from very low birth weight preterm infants. Uropathogens colonizing the vagina by may be a critical event preceding UTIs and may contribute to preterm birth (Kalyoussef et al., 2012). A study involving over 13,000 pregnant women for Vaginal Infections and Prematurity Study showed that an increase in *E. coli* or *Klebsiella pneumoniae* in vaginal cultures at delivery was associated with an increased risk of preterm birth and a higher adjusted odds ratio for preterm birth than any other factor (Sekwati-Monang et al., 2012).
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Hsieh et al reported that the viability of *H. pylori* was effectively suppressed by *Lactobacillus johnsonii* MH-68 and *L. salivarius subsp. salicinius* AP-32, and when used as probiotics, they may help decrease the occurrence of gastritis, and even reduce the risk of *H. pylori* infection (Hsieh *et al.*, 2012).

Spent Culture Supernatant (SCS) from a probiotic strain of *Lactobacillus, L. kefir*, which contain significant amounts of S-layer protein, inhibit *Salmonella* invasion, implicating the S-layer protein in host defense (Golowczye *et al.*, 2007). About 25% of the *Lactobacillus* SCS tested for antibacterial activity against Bacterial Vaginosis (BV) associated bacteria inhibited the growth of *Prevotella bivia, G. vaginalis and Mobiluncus spp* (Matu *et al.*, 2010). The bactericidal activity of filtered SCS of *L. jensenii* were found to be bactericidal for *E. coli* and persisted over a pH range of 4.3–6.3, reflecting the pH of MRS and LB broth, respectively (Kalyoussef *et al.*, 2012).

Studies have focused on the antimicrobial activity of hydrogen peroxide, lactic acid, and bacteriocins as mediators of the antimicrobial activity of *Lactobacilli*. Bacteriocins are proteins produced by non-pathogenic bacteria that inhibit the growth of competing strains. The activity of the bacteriocin in a study was shown to be lost when treated with proteinase K, suggesting that bacteriocins, which are proteinase K sensitive, contributed to the antimicrobial activity against the BV-associated bacteria (Kalyoussef *et al.*, 2012; Matu *et al.*, 2010).
Clostridium difficile is a spore-forming, toxin-producing, anaerobic bacterium that colonizes the human gastrointestinal tract. This pathogen causes antibiotic-associated diarrhea and colitis in animals and humans. Antibiotic-associated diseases may be treated with probiotics, and curiosity is increasing in such uses of probiotics. An investigative study was taken up by Yun et al. with an intention to experience the effect of Lactobacillus strains on the quorum-sensing signals and toxin production of C. difficile. In addition, an in vivo experiment was designed by them to assess whether Lactobacillus acidophilus GP1B was able to control C. difficile-associated disease. Autoinducer-2 activity was measured for C. difficile using the Vibrio harveyi coupled bioluminescent assay. Cell extract (10 μg/mL) of L. acidophilus GP1B was shown to have the highest inhibitory activity among 5 to 40 μg/mL cell-extract concentrations. Transcriptional level expressions of luxS, tcdA, tcdB, and texR genes were found to be reduced in the presence of 10 μg/mL of cell extract of L. acidophilus GP1B. Survival rates at 5 d for mice given the pathogen alone with L. acidophilus GP1B cell extract or L. acidophilus GP1B were also found to be increased. In addition, the lactic acid-produced L. acidophilus GP1B displayed an inhibitory effect against the growth of C. difficile. The results from this particular study put forward that L. acidophilus GP1B and GP1B cell extract have significant antipathogenic effects on C. difficile (Yun et al., 2014).

Many different mechanisms are employed by the pathogenic bacteria to delay wound healing such as consistent production of inflammatory mediators or maintenance of necrotic neutrophils, which release cytolysic enzymes and free oxygen radicals. Pseudomonas aeruginosa is one of the most common pathogens isolated from
infections in chronic wounds. This bacterium is awfully noncompliant to therapy and to host immune attack when it forms biofilms. Consequently, antibiotics and antiseptics are increasingly being rendered useless in the treatment of these infections. Lactobacillus plantarum had been reported to exhibit an important antipathogenic capacity on P. aeruginosa. Alberto et al. examined the effects of L. plantarum supernatants on pathogenic properties of P. aeruginosa, such as adhesion, viability, virulence factors, biofilm formation, and quorum sensing signal expression. L. plantarum supernatants were able to constrain pathogenic properties of P. aeruginosa by a quorum quenching mechanism. The antipathogenic properties mentioned above, together with the immunomodulatory, tissue repair, and angiogenesis qualities in the supernatants of L. plantarum, make them an attractive option in infected chronic wound treatment (Alberto et al., 2012).

2.6 Immune modulation and Lactobacilli:

Health claims of lactic acid bacteria (LAB) used in functional foods and pharmaceutical preparations are based on the capacity of these microorganisms to stimulate the host immune system (Perdigón et al., 2002). The mucosal immune system is responsible for 60% of the daily production of immunoglobulins. The cytokines released by the associated immune cells, may favour either a Th1 immunogenic response, a Th2 humoral or tolerogenic, or only an inflammatory response (Mestecky et al., 1987; Gonnella et al., 1998).

Different mucosal immune responses, specific or non-specific, by lactic acid bacteria (Vintin'I et al., 2000) was suggested to be due to the different pathways of internalization of LAB in the gut enabling these bacteria to induce different immune
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responses. LAB can make contact with the immune system associated to the intestinal mucosa through M cells or follicle associated epithelium (FAE) cells from Peyer’s patches or through the epithelial cells of small or large intestine (Perdigón and Oliver, 2000; Neutra and Kraehen, 1992). These findings could explain the diversity of induced mucosal immune response. The interaction of LAB with M cells induces mainly specific immune responses (Campbell et al., 1999), while the interaction with FAE cells induces a non-specific or inflammatory response, even though this mode of entry can also enhance the specific immune response. The interaction with epithelial cells can lead to enhancement of local immunity or non-response by antigen clearance (Hershberg and Mayer, 2000; Sudo et al., 1997). These studies have also demonstrated the importance of the ecological niche of the probiotic microorganism to elicit a better immunological effect on the small or large intestine (Perdigón et al., 2002; Hamilton-Miller et al., 2003).

The antigenic effect of LAB (Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus delbrueckii ssp. bulgaricus and Streptococcus thermophilus) on the gut immune system of BALB/c mice was evaluated and a dose-dependent increase of the Bcl2 protein was observed with all LAB assayed. Furthermore, the analysis of cytokine-producing cells in the lamina propria of gut showed that TNF-α and INF-γ values, determined in macrophages cultured from Peyer patches, were enhanced for the entire LAB assayed. An important increase of interleukins, IL-10 and IL-4, was observed mainly in mice fed with Lactobacillus delbrueckii ssp. bulgaricus or Lactobacillus casei, while a significant induction of IL-2 and IL-12 was only observed with L. acidophilus (P <0.01). It was demonstrated that orally administered
live LAB can modulate the systemic immune response according to the elicited cytokine profiles, and that the immune response obtained is highly related to the dose administered (Perdigón et al., 2002).

Murine models suggest that bacterial gut colonization is essential for postnatal maturation of Th1 immune responses and induction of oral tolerance (Sudo et al., 1997). Temporary colonization of the gut with an appropriate probiotic strain not only promotes the state of ‘eubiosis’ (favourable balance of the gut flora) but also can have a favourable immunomodulatory effect (Hamilton-Miller et al., 2003). However, the specific microbes or groups of microbes responsible for this phenomenon have not been confidently identified. In neonate mice, the administration of antibiotics leads to alterations of the intestinal flora and impaired Th1 immune responses that can be reversed by administration of Enterococcus faecalis as well as Lactobacillus acidophilus to neonates but not older mice (Sudo et al., 2002).

Neonatal treatment with Lactobacillus rhamnosus GG have been shown to inhibit the development of experimental asthma in mice that was associated with increased Foxp3 expression and TGF-β production (Feleszko et al., 2007). In another study, oral treatment with live Lactobacillus reuteri significantly attenuated inflammatory cell influx to the lung and decreased allergen-induced airway hyper-responsiveness in mice. In a follow up study, Forsythe and his group demonstrated that the Lactobacillus reuteri-induced attenuation of allergic airway response was mediated
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through the suppressive function of regulatory T cells (Forsythe et al., 2007; Karimi et al., 2009).

In other murine models, stimulation with LPS increased proliferation and efficiency of Tregs through activation of their Toll-like receptors (Caramalho et al., 2003; Mazmanian et al., 2005). In vitro experiments show that cultured human intestinal cells produce TGF-β in response to stimulation with microbial antigens (Yoshioka et al., 2001) and that some bifidobacterial species stimulate production of IL-10 in cord blood (Young et al., 2004).

Lactobacillus acidophilus, a natural inhabitant of the intestine, induces high levels of IFN-β in dendritic cells, thereby protecting the host from infection and boosting the immune response. After exposure to probiotic conditioned media (PCM), immature human enterocytes, immature human intestinal xenografts and primary enterocyte cultures of NEC tissue (NEC-IEC) were assayed for an IL-8 and IL-6 response to inflammatory stimuli as well as for innate immune response gene expression. In the immature xenograft, PCM exposure significantly attenuated LPS and IL1-β-induced IL-8 and IL-6 expression, decreased TLR2 and TLR4 mRNA, and increased mRNA levels of specific negative regulators of inflammation, SIGIRR and Tollip. In NEC-IEC, PCM decreased TLR2-dependent IL-8 and IL-6 induction and increased SIGIRR and Tollip expression (Ganguli et al., 2012).

Several in vitro studies show that Lactobacilli or their cell-free cultures inhibit or kill H. pylori, prevent its adhesion to mammalian epithelial cells and prevent IL8 release.
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In vivo models demonstrate that pre-treatment with a probiotic can prevent *H. pylori* infections and/or that administration of probiotics markedly reduced an existing infection (Hamilton-Miller, 2003).

Murine helper T cells are mainly classified into two categories according to the pattern of cytokine production displayed. Type 1 helper T cells, Th1 cells, produce IFN-γ, IL-2 and TNF-β, whereas Type 2 helper T cells, Th2 cells, produce IL-4, IL-5, IL-6 and IL-10. The Th1 cytokines augment cellular immunity and Th2 cytokines augment humoral immunity (Mosmann and Coffman, 1989; Mosmann et al., 1986). The balance of the two cell populations is believed to be important for the maintenance of homeostasis in the host. Once this balance becomes disturbed, various immunological diseases, such as allergies and infections, can occur through the evasion of host defence mechanisms. In recent years, beneficial effects of probiotics on the immune mediated diseases, such as allergy and asthma, have been documented. It has therefore been suggested that probiotic bacteria can also be used in the treatment of food allergies (Wheeler et al., 1997).

One possible mechanism for the beneficial effect of probiotics on allergic responses is to exert an inhibitory influence on the production of IgE (Shida et al., 1998). It is not clear whether oral administration of these probiotics can inhibit IgE production in vivo. This has been investigated by feeding heat-killed LcS to BALB/c mice. The mice were then immunized by intraperitoneal injection of ovalbumin (OVA) and Al(OH)3 on days 0 and 14. Seven days after this, blood was collected from all mice and assayed for OVA-specific serum IgE, while spleen cells were prepared for assays.
of OVA-specific IgE production and OVA-induced cytokine production (Takeshi Matsuzaki and James Chin, 2000).

Mice fed a diet containing 0.05% (w/w) LcS had a significantly reduced level of OVA-specific IgE compared with non-probiotic controls. In addition, in mice fed LcS, the level of production of Th1-associated cytokines, such as IFN-γ and IL-2, by spleen cells was higher than that in the control group. In contrast, the level of production of Th2-associated cytokines, such as IL-4, IL-5, and IL-6, by spleen cells from the mice fed LcS was lower than that of the control group. Furthermore, levels of IL-12, which augments IFN-γ production by spleen cells from the mice fed LcS, were also higher than those of the control group. As mentioned earlier, some probiotics may have the potential to ameliorate host immune system disorders via. These results indicate that LcS feeding induces a Th1 rather than a Th2 response. Therefore, we propose that augmentation of Th1 cells by feeding LcS provides a way to inhibit Th2 cells and thereby reduce the production of IgE, regulating the Th1 and Th2 balance. We believe that it is now time for researchers to focus more research on probiotics for the treatment of immune-mediated diseases, including allergies (Takeshi Matsuzaki and James Chin, 2000).

Interleukin-10 (IL-10) is a pluripotent cytokine and the most significant anti-inflammatory cytokine found within the human immune response (Alejandra et al., 2011; Asadullah et al., 2003). IL-10 was first described as a product of T-helper type 2 (Th2) cells that inhibited cytokine synthesis in Th1 cells, and receiving as such the designation of cytokine synthesis inhibition factor (CSIF) (Lalani et al., 1997;
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Howard and O’Garra, 1992; Opal et al., 1998). IL-10 is now known to be produced by numerous immune cell populations such as macrophages, monocytes, dendritic cells (DC), B cells, as well as Th2, Th1, CD4+CD25+ naturally occurring regulatory T cells (nTreg), Tr1 and CD8+ cells (Moore et al., 2001; Kamanaka et al., 2006) and as such, modulates the function of several adaptive immunity-related cells. IL-10 can induce development of IL-10-producing T cells by acting on antigen-presenting cells (APCs) such as dendritic cells and/or directly on the Treg cells. Tolerogenic DC can influence the development and activity of regulatory T cells. Furthermore, receptors for IL-10, IL-10R1, and IL-10R2, are expressed on many cell types and can also be observed in IL-10-producing cells, suggesting that the IL-10-secreting cells can themselves also be targets. However, IL-10 is also produced by nonimmune cell sources such as keratinocytes, epithelial cells, and even tumor cells (Alejandra et al., 2011; Moore et al., 2001; Williams et al., 2004).

Human IL-10 is a homodimer with a molecular mass of 37 kDa and each monomer consists of 160 amino acids. The human IL-10 gene is located on chromosome 1 and encodes for five exons. Human and murine IL-10 sequences exhibit approximately 80% homology. IL-10 exerts a profound effect on immune responses having the ability to differently affect the function of several immune cell subsets and is, therefore, considered to be a broad effector molecule in immunoregulation / host defense. IL-10 is generally considered an immunosuppressive molecule with its main biological function being the limiting and termination of inflammatory responses and the regulation of differentiation and proliferation of several immune cells such as T cells, B cells, natural killer cells, APCs, mast cells, and granulocytes(Asadullah et al., 2003; Mocellin et al., 2004).
However, IL-10 also mediates immunostimulatory properties in several *in vitro* and *in vivo* models (Mocellin *et al.*, 2004). The balance between both immunostimulatory and immunosuppressive effects is greatly influenced by the dominant cell function determining a given immune phenomenon. Thus, IL-10 can directly regulate innate and adaptive Th1 and Th2 responses by limiting T cell activation and differentiation in the lymph nodes as well as suppressing proinflammatory responses in tissues, leading to impaired pathogen control and/or reduced immunopathology (Couper *et al.*, 2008). As such, all the activities of IL-10 affects the inflammatory or specific cellular immune response (Th1, cytokine pro-inflammatory secretion by macrophages, and modulation of Th2) and stimulates functions of innate immunity (NK cell activity, non-inflammatory removal of particles, cells, and microbes by stimulating phagocytosis) and of Th2 related immunity. Thus, it inhibits the production of pro-inflammatory mediators while enhancing the production of anti-inflammatory mediators (Asadullah *et al.*, 2003). IL-10 has an effect on survival, proliferation, and differentiation of human B cells as well as inducing IgA and IgG production by B cells. Regarding the effects on T cells, IL-10 inhibits the production of IL-2 and IFN-γ by Th1 cells (Del Prete *et al.*, 1993; de Waal *et al.*, 1991) and decreasing T cell-mediated immunity while enhancing humoral immune response. IL-10 also promotes antigen uptake by DC (Allavena *et al.*, 1998; Morel *et al.*, 1997), inhibits DC migration (Demangel *et al.*, 2002; Wang *et al.*, 1999) and increases the expression of toll like receptors (TLR) on monocyte lineage cells (Flo *et al.*, 2001; Vabulas *et al.*, 2002).
Moreover, IL-10 also stimulates NK cell cytotoxicity (Mocellin et al., 2004; Shibata et al., 1998). The effects of IL-10 on immune cells suggest that the major physiological importance of IL-10 seems to be the limitation of inflammation, prevention of uncontrolled unregulated immunological reactions as well as the support of the humoral (Th2) immune response (de Waal et al., 1991). The powerful immunomodulatory properties of IL-10 and the promising results from IL-10 delivery on the course of several inflammatory diseases in experimental models induced the interest on clinical application of IL-10. However, inadequate IL-10 expression seems to have a considerable pathological impact. Both overexpression (e.g., in lymphoma) as well as IL-10 deficiency (e.g., in inflammatory bowel disease) are likely to have a physiological significance. Therefore, neutralization of the cytokine could be a promising approach to treat diseases from the first group whereas application of IL-10 itself could be helpful for diseases from the second group (Asadullah et al., 2003). It is believed that the reliable manipulation of immune responses by controlling IL-10 expression in the cellular location where it is produced may someday become reality (Mosser et al., 2008). In this context, we can say that currently there is being a continuous expansion of knowledge of one of the mechanisms by which LAB and other probiotic microorganisms participate in the prevention and treatment of gastrointestinal inflammatory disease through their immune-modulating properties with special emphasis on the critical role of the anti-inflammatory cytokine IL-10 (Alejandra et al., 2011).

The interleukin 4 (IL4) is a cytokine which encourages differentiation of naive helper T cells (Th0 cells) to Th2 cells. Upon stimulation by IL-4, additional IL-4 is
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subsequently produced by Th2 cells. The cell that initially produces IL-4, thus
inducing Th0 differentiation, has not been identified, but recent studies suggest that
basophils may be the effector cell (Sokol et al., 2008). It is closely associated with
functions being quite similar to Interleukin 13. The receptor for Interleukin-4 is
identified as the IL-4Ra. This receptor occurs in 3 different complexes all through
the body. Type 1 receptors are composed of the IL-4Ra subunit with a common γ
chain and specifically bind IL-4. Type 2 receptors comprise of an IL-4Ra subunit
bound to either another IL-4Ra, or a different subunit known as IL-13Ra1. These
type 2 receptors have the capacity to bind both IL-4 and IL-13, two cytokines with
closely connected biological functions (Maes et al., 2012; Chatila et al., 2004).

Interleukin 4 is associated with many biological roles, comprising the stimulation of
activated B-cell and T-cell proliferation as well as the differentiation of B cells into
plasma cells. It is a crucial regulator in humoral and adaptive immunity. IL-4 induces
B-cell class switching to IgE, and up-regulates MHC class II production. IL-4
reduces the production of Th1 cells, macrophages, IFN-gamma, and dendritic cell
IL-12, while overproduction of IL-4 may result in allergies (Hershey et al. 1997). IL-
4 also has been reported to drive mitogenesis, dedifferentiation, and metastasis in
rhabdomyosarcoma (Hosoyama et al., 2011).

An important role is played by tissue macrophages in chronic inflammation and
wound repair. The extravascular tissue presence of IL-4 stimulates alternative
activation of macrophages into M2 cells and impedes classical activation of
macrophages into M1 cells. An upsurge in repair macrophages (M2) is coupled with
secretion of IL-10 and TGF-β that affect the decrease of pathological inflammation.
Release of arginase, proline, polyaminases and TGF-β by the activated M2 cell is strung together with wound repair and fibrosis (Jon et al., 2009).

Orally administered *Lactobacillus plantarum* PM008 potently inhibited the expression of IgE-switching cytokine, IL-4, and of proinflammatory cytokines, IL-1β and TNF-α, in the colon of mice. Its inhibitory effect was dependent on the dosage and administration period (Jang et al., 2011).

One of the studies found a remarkable effect for the production of anti-inflammatory cytokines such as IL-4 or IL-10. The high levels detected for IL-4 and the knowledge that Th1 cells stimulate IgG2a antibodies production, whereas IgG1 antibodies are induced under control of Th2 (cytokine IL4), led the researchers to analyse the antibody isotype against ovoalbumin induced after oral administration of LAB. It was then shown that *L. casei*, *L. delbrueckii ssp. bulgaricus* and *L. acidophilus* affect the systemic humoral immune response. This finding was then proposed to be considered in the selection of *Lactobacilli* strains as vectors for oral vaccine which would alleviate the negative aspects of gut inflammation by some oral vaccines (Perdigón et al., 2002).

Several observations lead to the notion that enhanced secretion of Th1-type cytokines, such as IL-2 and IFN gamma , and TNF alfa , acts as a key factor in the pathogenesis of Crohn’s disease. Additionally, IFN gamma mainly augments cellular immunities and exhibits antitumor responses, and it inhibits the production of Th2-type cytokines such as IL-4. Plant lectins like Con A treatment to probiotic treated
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murine spleen cells have been shown to increase the production of IFN gamma production (Chia-Yang et al., 2011).

Several reports have shown that probiotics skew the Th1/Th2 balance toward Th1 by increasing the production of Th1-type cytokines such as IFN gamma in monocytes, dendritic cells, macrophages, and PBMCs after cells have been incubated with several strains of Lactobacillus or Bifidobacterium (Kanzato et al., 2008; Isolauri et al., 2001; Kato et al., 1999; Matsuzaki). Furthermore, the effects of Lactobacillus or Bifidobacterium on the stimulation of Th1 immunity have also been reported in clinical trials. In addition, spleen cells from mice given L. acidophilus also produced significantly higher amounts of IFN gamma in response to stimulation with ConA compared to cells from the control mice. Studies have indicated that L. casei strain Shirota feeding induced a Th1 response rather than a Th2 response (Kato et al., 1999; Matsuzaki et al., 2000).

The amount of interferon gamma produced murine splenocytes when cocultured with LAB was shown to vary according to concentration and strain of the LAB. It was also found in a particular study that the probiotic strain used in that study promoted a Th1 response in mice and humans. On the other hand, the increased production of IFN gamma suggested that this cytokine may be an important factor in enhancing the cellular immunity and inhibition of cancer cell proliferation that have been observed by many research groups (Chia-Yang et al., 2011).
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The level of production of Th1-associated cytokines, such as IFN-γ and IL-2, by spleen cells was higher than that in the control group in mice fed with *Lactobacillus casei* strain Shirota (LcS). It was proposed that increase of Th1 cells by feeding LcS provides a way to inhibit Th2 cells and thereby reduce the production of IgE. The study tried to demonstrate that promoting a bias towards a Th1 response certain specific IgE production by treatment with LcS suggesting at least some merit in exploring the potential of probiotics in the treatment of allergic disorders (Takeshi Matsuzaki and James Chin, 2000).