The Role of the Immune System in the use of Probiotic Lactic Acid Bacteria in Preventing and Treating Allergic Diseases

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Abstract
The immune system is generally divided into the innate and the adopted immune systems, both protecting the body from pathogens. Recently, allergies, a disease associated with an imbalanced immune system, have increased rapidly in developed countries. Prevailing symptoms of allergic diseases are eczema, allergic rhinitis, asthma, inflammatory bowel disease, and food allergy. Probiotic bacteria, mainly consisting of lactic acid bacteria, are used in the prevention and treatment of allergic diseases. The function of them is to stimulate the intestinal immune cells and form a complex signal network to activate other immune cells. Beneficial health effects of probiotics are based on the hygiene hypothesis, which suggests that sanitary environment is important for health, but limited exposure to environmental factors increases allergic diseases. An immunoregulatory effect of probiotic bacteria is demonstrated by controlled trial, animal model, in vitro, in vivo and ex vivo designs. However, the immunoregulatory effect of probiotic bacteria is controversial because it depends on probiotic strains, a dose and a type of diseases. In this review, we discussed clinical evidences on immunoregulatory effects of probiotic bacteria.

Key words: immunoregulation, allergic disease, probiotic bacteria, LAB, gut microflora

Introduction
A new disease is increased recently with the development of industry and economy. Allergy is a symptom that causes the hypersensitivity to a specific person induced by allergens, which are not reacted to the common people. Basically, most allergic reactions are a hypersensitivity against the specific antigen called allergen (Jeong et al., 2007) and are defined as an imbalance of the lymphocyte-mediated immune response by over-responses between the allergic molecules and lymphocytic Th2 cells. In other words, an allergen-sensitive Th2 cell produces cytokines such as IL-4, IL-5 and IL-13, and these lymphocyte cells then promote allergic and atopic symptoms either alone or along with IgE produced from B cell. Especially, an atopic disease afflicts neonate and young children at an exponential rate in developed countries. Food allergies and atopic eczema are the most common atopic symptoms in infancy (Gustafsson et al., 2000; Zeiger, 2003). Although the atopic symptoms appear in infancy clearly, it may originate in the fetus. The occurrence of food allergies can be considered the lack of immune maturation rather than the reduction of tolerance to an allergen. Thus, the measures for the reduction of atopic diseases must originated from the perinatal period (Rautava, 2007).

Recent rapid increases in allergic and atopic diseases has occurred in developed countries. In particular, their incidence is increasing in cities and in wealthy and small families in comparison to larger, poor, rural families in developing countries. The reason for the rise of allergies has not been clearly identified yet, but widely accepted ideas are environmental and dietary factors, an introduction of new allergens and a reduced exposure to pathogens (Jeong et al., 2007).

Intestinal microflora established in early infancy after birth affects the physiological condition of the host, i.e., essential nutrients, metabolism of indigestible substances, inhibition of colonization by pathogens and the establishment the intestinal structure (Borchers et al., 2009; Hooper et al., 2000, 2002). In addition, intestinal microflora is associated with the development of the immune system and the incidence of allergic diseases (Tang, 2009). Above all, appropriate gut microflora composition improves the function of the intestinal track, resulting in
lower intestinal inflammation and improved barrier function may be established. The hygiene hypothesis suggests that early infancy infections could suppress the Th2 cell response by improving immune responses, so that the intestinal microflora plays an important role for maturation of immune system (Kalliomaki et al., 2001; Kan-kaanpaa et al., 2002).

Probiotic bacteria, living in the intestinal track of humans and other mammalians, offer the beneficial health effects on the host. The main roles of probiotic bacteria in the gut are the protective effect by immune function and controlling gut microflora between useful and pathogenic microorganisms (Oelschlaeger, 2009). According to the hygiene hypothesis, probiotic bacteria are used in the prevention and treatment of allergic disease in early infancy (Kalliomaki et al., 2001). However, the effect of probiotic bacteria on the immune function has still remains in controversy. Therefore, the objective of this review was to introduce the recent trend of prevention and treatment of allergic diseases by fermented milk products containing probiotic lactic acid bacteria.

Functions of Immune System

Human and other mammalian offer a great many regions that can be colonized by microflora such as skin the intestinal track, and the respiratory track. Microbial colonization on the host has two opposed effects of the adaptability of microflora. Intestinal colonization can provide health benefits, including maintaining intestinal homeostasis (Rakoff-Nahoum et al., 2004) and immunoregulatory effects, while the colonization of pathogens can induce serious diseases (Medzhitov, 2007).

The main function of the immune system is to prevent the microorganisms, such as viruses, microflora and fungi, to protect the body from pathogenesis agents. There are two immune mechanisms in the human body; the innate and adaptive immune systems. The recognizing mechanism of the innate immune response consisting of physical barriers, such as mucous membranes, the phagocytic and cytotoxic function of neutrophils, monocytes, macrophages and lymphatic cells (NK cells), is based on the pattern-recognition receptors (PRRs), which have wide specificities via the stored microorganism’s profiles (Janeway, 1989). When the pathogens are detected by PRRs, B cells release antibodies to attack them. T cells can become many different types depending on their functions. T cytotoxic (Tc) cells are used for protecting the body from the attack of microorganisms, viruses or cancer cells. Meanwhile, T helper (Th) cells play an important role in activating B cells (Lim et al., 2009).

PPRs can bind with a plenty of specific molecules that have the same patterns that they conserve. They do not separate the pathogen from commensal bacteria, which is important in maintaining gut homeostasis (Rakoff-Nahoum et al., 2004). Toll-like receptor (TLR), one of the pattern-recognition receptors, recognizes the microbial substance and prevents them. TLRs detect viruses, microbacteria or their products and induce an inflammatory reaction.

On the other hand, the recognition of an adaptive immune response is related to antigen receptors, such as B cells and T cells, which have random and narrow specificities, and is not increased by repeated exposure (Schatz et al., 1992). When microbial substances are presented by antigens presenting cells in the peripheral tissue and transferred to lymph nodes or spleen cells, those are indicated by lymphocyte, such as B-lymphocytes and T-lymphocytes, which are expressed into appropriate types.

B cells activated by T cells protect the body from microbial invasion via producing antibodies, such as IgA, IgE, IgG, IgM and IgD. On the other hand, T cells directly or indirectly affect the immune response. For example, when T helper cells detect invaders and infected cells, they produce signals to activate T cytotoxic cells as well as other immune cells, including B cells, and directly eliminate invaders and infected cells (Hirschhorn-Cymerman et al., 2009). Consequently, the immunoregulatory response of each immune system is based on the multiplicity of strains with different modes.

Intestinal Microflora and Gut Functions

Mammalian have $10^{12}$ CFU/g of intestinal microflora consisting of more than 500 numbers of microbial strains (Hooper and Gordon, 2001). Researchers have suggested that these intestinal microflora are helpful to hosts, and some of those microflora have been used as probiotic strains (Guarner and Malagelada, 2003; Rastall, 2004).

Interaction between gut microflora and the host affects the physiological state of both participants. Gut microflora participate in various responses in the gut, i.e., neutralizing the colonization of pathogens, modifying the microbial composition, improving the intestinal barrier function and activating the mucosal immune cell (Hooper et al., 2000, 2002; Mountzouris et al., 2009).

Gut mucosa consisting of an important interface between gut environment and host promotes nutrient absorption
while prevent the invasion of pathogens. GALT (gut-associated lymphoid tissue) is the largest tissue among lymphoid tissues in the body, which is extremely exposed by antigens produced from gut microflora during its colonization. The composition of colonizing microflora depends on the mode of birth, diet and hygiene levels. The role of gut microflora is essential to mature and maintain the immune response (Borchers et al., 2009). Cumulative evidence has maintained that an interaction between the intestinal microflora and toll-like receptors is important to maintain gut homeostasis (Rakoff-Nahoum et al., 2004).

**Effects of Probiotic Bacteria on Immunoregulation**

Most bacteria penetrate the body through the oral route. When the antigen goes into the body, lymphoid cells with mucosa produce systemic immune stimulation, which interacts with the antigen. Then, the determination non-specific or specific immune response is implemented through phagocytic activity of the peritoneal macrophages and the activation grade of T-lymphocytes by assays of delayed type hypersensitivity. When LAB is taken into the body through the mouth, the survivability of them depends on the pH, bile acid, and proteolytic enzymes (Ouwehand et al., 2002; Pan et al., 2009).

Some health benefits of probiotic bacteria include prevention of infectious diseases and food allergies, improvement of the normal microflora, stabilization of the gut mucosal barrier, and improvement in the digestion of lactose in intolerant hosts (Alander et al., 1999; Parvez et al., 2006; Quigley, 2007). Some bacteria, including Lactobacillus, Streptococcus, Bifidobacterium, Lactococcus and some fungal strains, such as Saccharomyces and Boulandii, are commonly used as probiotic strains as well as dead cells (Vanderhoof, 2008). The most commonly used probiotics are lactic acid bacteria (LAB), mainly Lactobacillus and Bifidobacterium strains. They are good for health via promoting disease resistance (de Waard et al., 2001; Weizman et al., 2005) and quelling intestinal diseases and allergies (Cross et al., 2001; Isolauri, 2001). Fermented milk, cheese, fruit juices, wine and sausages are foods which contain a great deal of LAB. The effect between viable and dead cells on the immunoregulation is sometimes the same and sometimes different due to differences among their composition, important factors and released products. Furthermore, a large number of same microbial strains differ in their structural properties and released substances. Plenty of studies have suggested that the power to induce cytokine production and a signal molecule activating other immune cells depends on the microbial cell wall composition (Helwig et al., 2006; Medina et al., 2007; Shida et al., 2006).

Some studies have indicated that LAB must remain viable in order to maintain effects on the immune system, but it is true only for some strains (Galdeano and Perdigon, 2004). Simultaneously, to achieve optimal effects, LAB must remain in the gut at least 48 to 71 h. During milk fermentation in the presence of LAB, various biologically active metabolites or compounds are released in the medium. These products can inhibit enzyme activities and prevent cancer, i.e., β-glucuronidase and nitroreductase (de Moreno de LeBlanc and Perdigon, 2005).

In mice, different types of LAB were used to lead the increasing of con-cavallin A and lipopolysacharide, which are created by spleen cells. These cells also produce significantly higher amounts of interferon-γ in response to stimulation with concavalin A (Gill et al., 2000). In the gut local immunoglobulin A (IgA) is generated and increased by an immune response, which is stimulated by bacteria (Milling et al., 2005) or fermented milk such as yogurt (Perdigon et al., 2002). While the stimulation with probiotic bacteria induced on the epithelial and immune cells that different patterns of cytokine in the intestine depends on the amount of LAB (Vinderola et al., 2005) (Table 1).

Physiological processes are decreased by the senescence of the immune system. With recorded declines in T helper cell (Th) responses, the cell-mediated immunorespnsae is most noted (e.g., ability to secrete IL-2 in vitro). Alternatively, there are two important points that the probiotic-mediated increases in ex vivo NK (natural killer) cell tumoral activity are significantly correlated with age (Gill et al., 2001b) and the individuals with the poorest pretreatment immunoresponses were the ones who experienced the greatest relative increases in the cell function.

In some studies, some strains of LAB are effective in reducing the incidence of cancer and infectious diseases, ameliorating inflammatory bowel diseases, and preventing allergies in experimental animal models and in humans (Kalliomaki and Isolauri, 2004; Rafter, 2002; Sartor, 2004). Milk with Lactobacillus GG has prevented the usual hypersensitivity reaction in people with milk allergies. Stimulation of immunity has been observed in clinical trials (Table 2).
Experimental Evidences for Effect of Probiotic Bacteria

The beneficial immunological effect of fermented milk products containing probiotic bacteria on the health has studied through animal trials, in vitro and ex vivo design (Adolfsson et al., 2004) as well as randomized placebo-controlled trial (Kekkonen et al., 2008). Some studies reported that probiotic bacteria may decrease the allergic symptoms, such as asthma, eczema (Isolauri et al., 2000), allergic rhinitis and food allergies (Salmi et al., 2009) during infancy. The hygiene hypothesis supports these evidences, which indicates that reduced infections from environmental factors in infancy may increase allergic diseases (Wichers, 2009). An immune system is adopted after birth and is matured by exposure to the environment. That is, exposure only to a hygienic environment decreases the opportunity to activate immune cells. In consequence, an immature immune system may induce allergic diseases when stimulation from antigens.

Some studies have demonstrated that the yogurt containing probiotic bacteria, such as Lactobacillus and Bifidobacterium, can provide beneficial health effects (Adolfsson et al., 2004; Baba et al., 2006) by maintaining the balance of the intestinal microflora between the useful and pathogenic bacteria (Ouwehand et al., 2003) and improving immune functions (Aldinucci et al., 2002).

Aldinucci et al. (2002) announced that a yogurt supplemented fermented nasal inflammation patient group better reflected increased IFN-γ level and decreased IL-4 level in blood samples than a non-yogurt supplemented group. Moreover, de Moren de LeBlanc et al. (2008) showed that IgA-secretion in a weaning mice group fed from mothers that had consumed fermented milk containing a probiotic bacterium (L. casei DN-114001) increased more than a placebo group. Furthermore, Ana et al. (2009) announced that the consumption of fermented milk products containing probiotic bacteria, such as L. gasseri CECT5714 and L. coryniformis CECT5711 in children suffering from allergic disease for 3 mon decrease the IgE level operating mainly on allergic symptoms, while IgA, natural killer cell (NK cell) and CD4+/CD8+ T regulatory cell levels are significantly increased. Also, Kawase et al. (2009) suggested that yogurt consumption containing probiotic bacteria for 9 weeks reduces IL-4 and IL-5 levels in patients suffering seasonal allergic rhinitis. Likewise, a large number of studies have investigated the role of different probiotic strains on the immunoregulatory effect in a healthy individual. Some studies have been reported the increasing NK cell response through the consumption of L. casei Shirota (Morimoto et al., 2005; Nagao et al., 2000; Takeda and Okumura, 2007), L. rhamnosus HN001 (Gill et al., 2001a; Sheih et al., 2001), and B. lactis HNO19 (Chiang et al., 2000; Gill et al., 2001b). Other studies also proved that specific probiotic bacteria can increase the phagocytic response of phagocyte with consumption.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Feeding period (d)</th>
<th>Number of cells producing</th>
<th>Cytokines</th>
<th>IgA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>TNF-α</td>
<td>IFN-γ</td>
</tr>
<tr>
<td>L. casei CRL 431</td>
<td>2</td>
<td>90±8*</td>
<td>124±15*</td>
<td>24±6</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>74±10*</td>
<td>116±18*</td>
<td>28±7</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>52±7</td>
<td>85±19*</td>
<td>20±9</td>
</tr>
<tr>
<td>L. delbrueckii subsp. bulgaricus CRL 432</td>
<td>2</td>
<td>79±6*</td>
<td>59±23*</td>
<td>40±8</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>59±11</td>
<td>72±18*</td>
<td>42±8</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>43±12</td>
<td>209±34*</td>
<td>42±18</td>
</tr>
<tr>
<td>L. acidophilus CRL 724</td>
<td>2</td>
<td>52±7</td>
<td>51±25*</td>
<td>27±8</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>51±9</td>
<td>73±11*</td>
<td>25±7</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>22±11</td>
<td>64±6*</td>
<td>31±13</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>24±4</td>
<td>17±6</td>
<td>31±12</td>
</tr>
</tbody>
</table>

1The cytokine-producing cells and IgA-secreting cells were determined on histological slices from the small intestines of BALB/c mice by an immunofluorescence test. The animals were fed in their drinking water lactic acid bacteria (1×10^8 CFU/mL/d) for 2, 5, or 7 consecutive days. L. casei and L. acidophilus were isolated from human feces, and L. delbrueckii subsp bulgaricus was from yogurt. The animals received 2.5 or 3 mL/d.

2Three measurements were taken, and values are means±SD.

*Significant difference between test and untreated control groups (p<0.01).
Table 2. Overview of clinical trials assessing the effects of probiotic bacteria on the immune system

<table>
<thead>
<tr>
<th>Probiotic Allergy</th>
<th>N</th>
<th>Age and sex</th>
<th>Duration</th>
<th>Design</th>
<th>Effect of probiotic intervention</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yogurt with L. debrueckii bulgar-iccus, S. thermophilus, and L. acidophilus</td>
<td>Rhinitis</td>
<td>13 patients and 3 controls</td>
<td>19-44 years; 7 males, 6 females</td>
<td>Group 1: Rhinitis patients given yogurt with probiotics for 4 month (N=7) Group 2: Rhinitis patients given partial skim milk (N=6) Group 3: Healthy controls with no intervention (N=3)</td>
<td>Placebo-controlled, no information on whether randomized or double-blind, placebo controlled trial</td>
<td>Increased IFN-γ production in unstimulated PBMCs (not significant); decreased IL-4 production in PHA-stimulated PBMCs (not significant); improvement of mucociliary clearance (P=0.03); decreased symptomatological score (P=0.004); no effects on IgA or skin-prick test</td>
</tr>
<tr>
<td>Mixture of L. rhamnosus GG, L. rhamnosus LC705, B. breve Bb99, and P. freuden-reichii</td>
<td>Atopic Dermatitis with or without cow’s milk allergy</td>
<td>119</td>
<td>1.4-11.5 mon 61% male</td>
<td>Group 1: L. rhamnosus GG (N=42) for 4 wk Group 2: Mixture (N=41) for 4 wk Group 3: Placebo (N=36)</td>
<td>Randomized double-blind, placebo-controlled trial</td>
<td>L. rhamnosus GG increased IFN-γ production in stimulated PBMCs pre- and post-intervention (P=0.023) compared with placebo group (P=0.06); mixture increased IL-4 production in stimulated PBMCs in infants with atopic eczema and cow’s milk allergy pre- and post-intervention (P=0.034) compared with placebo group (P=0.028)</td>
</tr>
<tr>
<td>Mixture of L. rhamnosus GG, L. rhamnosus LC705, B. breve Bb99, and P. freuden-reichii spp. shermanii JS</td>
<td>Asthma, Allergic rhinitis, Atopic eczema</td>
<td>925 Infants</td>
<td></td>
<td>Group1: L. rhamnosus GG Group 2: L. rhamnosus LC705 Group 3: B. breve Bb99 Group 4: P. freudenreichii spp. shermanii JS for 6 mon Group 5: Placebo</td>
<td>Randomized double-blind, placebo-controlled trial</td>
<td>Probiotic bacteria groups have higher CRP (P = 0.008), total IgA (P = 0.016), total IgE (P = 0.047), and IL-10 (P = 0.002) levels than placebo group.</td>
</tr>
</tbody>
</table>
of a mixture of _L. acidophilus_ 74-2 and _B. animalis_ subsp. _lactis_ DGCC 420, and those strains mixture plus _L. paracasei_ Lpc-37 (Klein et al., 2008; Roessler et al., 2008). Meanwhile, another study announced that similar strains mixture consisting of _L. paracasei_ spp. _paracasei_ CRL-431 and _B. animalis_ spp. _lactis_ Bb-12 have no effect on the phagocytic response (Christensen et al., 2006). These results reflect that the immunoregulatory effect of probiotic bacteria depends on the strain and type of immune response.

Wheeler et al. (1997) suggested that early infancy probiotic bacteria consumption has no effect on improving immune functions. Recently, Dekker et al. (2009) established that there were no statistically significant positive effects among three infant groups with placebo, _L. rhamnosus_ HN001 and _B. animalis_ subsp. _lactis_ HN019 supplement groups. Another study achieved similar results with the above (Kopp et al., 2008).

However, in spite of reported evidence that early infection to a probiotic bacteria possess no effects on the immunoregulation, a widely accepted idea is that the implement of fermented dairy products, such as yogurt, containing probiotic bacteria can improve the immune system and the reduce allergic symptoms.

**Immunoregulatory Mechanism of Probiotic bacteria**

The mechanism of immune response regulation by probiotic bacteria has not been clearly established; however, various studies demonstrated the immunoregulatory effect of probiotic bacteria through laboratorial studies (Bickert et al., 2009; Kekkonen et al., 2008). A widely accepted idea is that probiotic bacteria has no direct effect on immune response (Cross et al., 2001), but it may stimulate the intestinal immune cells and increase the signals activating others.

Some studies reported that probiotic bacteria can stimulate the producing immunoregulatory factors from randomized placebo-controlled experiment (Viljanen et al., 2005) and _in vitro_ design (Pohjavuori et al., 2004; Prescott et al., 2005). Moreover, Marschan et al. (2008) proved that a low grade inflammation by C-creative protein can reduce an eczema by increased levels of immunoregulatory factors, such as IgE, IgA and IL-10.

The mechanism of probiotic bacteria on immunoregulatory effects has been investigated from _in vitro_ and _in vivo_ designs. However, the effect on immunoregulation in these experimental designs shows different patterns (Savilahti et al., 2008). Gut microflora in mammalian has various useful effects on the intestinal track, which inhibits the pathogen’s colonization and maintains the intestinal immune system as well as nutrient metabolism (Vanderhoof, 2008). Intestinal immune cells and epithelial cells intercept microorganism invasion of the gut, and an invader is removed by innate immune system immediately (Neutra et al., 2001). The mucosal immune system performs its function through forming complex signals between immune cells, such as epithelial cells, macrophage and dendritic cells, and gut microorganisms and antigens. Intestinal immune cells activated by probiotic bacteria increases IgA production, which may induce the interaction between dendritic cells and lymphocytic T cells as well as B cells (Galdeano et al., 2007). Perdigon et al. (2001) demonstrated the internalization pathway of probiotic bacteria, such as _L. bulgaricus_ CLR 423, 431, 728 and _S. thermophilus_ CRL 412, through _in vitro_ study. Consequently, the important role of probiotic bacteria on the immunoregulation is to mediate the signal formation activating other immune cells by the intestinal immune system stimulation. The main immunoregulative interaction between probiotic bacteria and immune cells in the gut is shown in Fig. 1.

Most allergic diseases are induced by an imbalance of the lymphocyte-regulated immune system with an extreme response between allergen and the activated lymphocytic Th2 cells (Akdis et al., 2004; Cross et al., 2001; O’Connell, 2004). Th1/Th2 cell interaction is related to various cytokines such as interleukin (IL)-4, IL-5, IL-10, IL-12, IL-13 and interferon (YU et al., 2008). IL-4, IL-5 and IL-13 produced by Th2 cells supply granulated effectors to an inflammatory region, these initial cells promote the allergic and atopic symptoms alone or along the IgE antibody. IL-10 suppresses not only phagocytic cell, T cell, NK cell but also IFN-γ, IL-12 release (Borish, 1998). Moreover, IL-4 and IL-13 promote the conversion of the immunoglobulin produced in B lymphocyte to IgE. IFN-γ, a kind of interferon, inhibits IL-4 expression and IgE conversion.

Furthermore, interferon-1 (IFN-α), known as a signal molecule promoting IFN-γ activity, can improve the Th1 cell function, whereas reducing IgE formation, and IL-12 promotes Th1 cell differentiation in initial immune response while suppressing the cytokine release from Th2 cell (Davoine et al., 2006). Typically, interferon and IL-12 present lower levels in atopic patients. In conclusion, probiotic bacteria can down-regulate the Th2 cell over-expression through promoting the expression and secre-
The Role of Immune System for Prevention and Treatment of Allergic Diseases

Assessment of Efficacy and Safety of Probiotic Bacteria

In the European Union, probiotics are regulated via the Novel Food Regulation (258/97/EC). In short, this regulation is only applied to strains that were not used before 1997 and concerns novel foods or food ingredients. Recently, only in Denmark it is required that the relevant authority is notified by the manufacturer prior to the use of new probiotic strains.

In France and the USA the safety and efficacy data of probiotic bacteria in food was published by Agence Francaise de Sécurité Sanitaire and the Food and Drug Administration (FDA), respectively. It can be used as an additive and considered “generally recognized as safe” (GRAS). The suggestion of the GRAS as a probiotic has been guarded by these organizations since 1985, and the good expert in food safety field (Feord, 2002; Wright et al., 2005). Simultaneously, evaluating new probiotic bacteria may be approved by the Food and Agriculture Organization (FAO) of the United Nations and the World Health Organization (WHO) may be useful in evaluating newly discovered probiotics (Fig. 2).

Three steps for assessing the safety of probiotics has been proposed by Salminen et al. (1998) (Fig. 3). First, it should be determined by the intrinsic properties of the strain (e.g., enzymatic properties). Second, safety and stability (e.g., survival in the gut) of the strain should be evaluated. Third, interactions between the strain and host are studied. In this step, either in vitro assays or animal models were used to evaluate some functional and physiological aspects of probiotic bacteria.

The immunomodulatory effects of probiotics depend on the probiotic strains (Salminen et al., 2009) and their specificity (Boyle et al., 2009) as well as types of diseases (Wickens et al., 2008). Therefore, assessment of immunomodulation effects by a panel of assays was
required. Finally, after a long period of use, probiotics clearly play an important role as an anti-allergen.

Recently, hygienic environment reduced a disease, such as cytotoxism, induced by pathogenic bacteria and virus infection, but allergy and atopy, a new disease, are increasing rapidly. It is well known that fermented milk products, such as yogurt, can improve the health function. There are various therapeutical measures to reduce allergic symptoms, a recent study focused on immunotherapy through fermented milk product consumption. The effect of fermented milk products on immunoregulation is related to probiotic bacteria. An immunoregulatory effect by probiotic bacteria originates from the formation of complex signal network. In other words, cytokine, an important signal molecule, activates gut immune cells and further the others. The activated immune cell stimulates the other immune system, such as phagocytic, dendritic, NK cells by cytokine-mediated signal complex. The prenatal and pronatal probiotic treatments on allergic and atopic disease have been studying via randomized placebo clinical trials, but the evaluation of efficacy are controversial due to the diversity of experimental designs, strains, the consumption amounts and time periods. Lee et al. (2008) announced that consumption of fermented milk products containing probiotic bacteria is more efficient in prevention than treatment of allergic and atopic
Collectively, the efficacy of probiotic treatment on allergic diseases is controversial, though numerous beneficial evidences exist. So, the identification of potent strains that have immunoregulatory effects and clarifying the relation between probiotic strains and food ingredients are important (Isolauri and Salminen, 2008) for food application, especially for infant formula.

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**References**

5. Ana, M. e.-C., Sierra, S., Lara-Villoslada, F., Romero, J.,