Beneficial effects of probiotic microorganisms. A review

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Abstract. The aim of the present study was to describe the beneficial health effects of probiotic bacteria, manifested by inhibition of growth of pathogen microorganism strains and that of their colonization of the gastrointestinal tract of humans. The primordial mode of action by which a probiotic eradicates a pathogen can be facilitated by the production of antimicrobial substances such as bacteriocins, organic acids, and hydrogen-peroxide. Lactic acid bacteria and pathogens compete for receptor sites at the intestinal surface. Competition for these receptors will diminish the opportunity for pathogenic colonization and thus protect the host from infection. Many of the probiotic effects are mediated via immune regulation, in particular by the control of the balance of proinflammatory and anti-inflammatory cytokines.

Key words and phrases: probiotic microorganism, lactic acid bacteria, pathogen, competition, immune system.
1 General characterization

Probiotics are defined as viable microorganisms, which, in sufficient numbers, alter the microbiota of a host body compartment and thereby exert beneficial health effects (Shida-Nanno, 2008). The use of probiotics in enhancing intestinal health has been proposed for many years. As recently revisited by the Joint Food and Agriculture Organization/World Health Organization: probiotic strains are defined as “live microorganisms that, when consumed in an adequate amount as part of food, confer a health benefit on the host”. Probiotic strains are considered non-pathogenic and safe (Servin-Coccoiner, 2003).

Lactic acid bacteria (LAB) are regarded as a major group of probiotic bacteria. LAB are usually described as Gram-positive microorganisms, devoid of cytochromes and preferring anaerobic conditions, but are aerotolerant, fastidious, acid-tolerant, and strictly fermentative, producing lactic acid as a main product. The most important genera are: Lactobacillus, Lactococcus, Enterococcus, Streptococcus, Pediococcus, Leuconostoc, and Bifidobacterium. Members of the LAB are usually subdivided into two groups based on their carbohydrate metabolism. The homofermentative group consisting of Lactococcus, Pediococcus, Enterococcus, Streptococcus, and some lactobacilli utilize the Embden-Meyerhof-Parnas (glycolytic) pathway to transform a carbon source chiefly into lactic acid. As opposed to homofermentors, heterofermentative bacteria produce equimolar amounts of lactate, CO$_2$, ethanol, or acetate from glucose exploiting phosphoketolase pathway. Members of this group include Leuconostoc, Weissella, and some lactobacilli (Vasiljevic-Shah, 2008).

2 Effect on lactose digestion

In humans, the most highly investigated aspect of probiotics in digestion is their compensation for lactase insufficiency. Numerous studies have shown that better lactose digestion occurs in lactose malabsorbers who consumed yoghurt rather than milk (de Vrese et al., 2001).

Two hypotheses suggest that this effect does not correspond to a replacement of endogenous lactase by bacterial β-galactosidase. The gastric emptying of yoghurt has been found to be slower than that of milk, probably because of parameters such as viscosity or pH independent of the presence of bacteria. This delayed passage of lactose would give the residual endogenous lactase activity in the small intestine more time to hydrolyze the lactose. The second hypothesis is based on the fact that colonic microflora contribute to lactose
degradation in lactose maldigesters. Since LAB can stimulate colonic bacterial activity, it has been suggested that the beneficial effects of yoghurt in lactose malabsorbers result from an improved digestion of lactose in the colon. Even if these two hypotheses cannot be excluded, bacterial $\beta$-galactosidase probably cleaves lactose into galactose and glucose in the small intestine (Fioramonti, 2003).

3 Competition with pathogens

Many mechanisms have been postulated by which probiotics could enhance intestinal health, including competition for limited nutrients, inhibition of the epithelial and mucosal adherence of pathogens, inhibition of epithelial invasion by pathogens, the production of antimicrobial substances and/or the stimulation of mucosal immunity (Servin-Coccoiner, 2003).

Some probiotic bacteria with beneficial health effects have also been noted to adhere to the intestinal mucosa (Figure 1), suggesting that adhesive probiotics can prevent the subsequent attachment of pathogens, a phenomenon known as competitive exclusion.

Figure 1: The mechanisms by which normal flora (probiotics) compete with gut pathogens (Lu-Walker, 2001)
The intestinal normal flora can enhance host defense by occupying the gut in large numbers and diversity, thus:

- preventing the colonization of the host by pathogens by competing for essential nutrients or epithelial attachment sites;
- producing antimicrobial compounds, volatile fatty acids, and modified bile acids that in turn create a luminal microenvironment unfavorable for the growth of pathogens;
- inducing recruitment of immune cells and activation of appropriate immune and inflammatory responses. Intestinal disease will occur when several factors that overcome these microenvironmental and immunologic responses disrupt the integrity of the epithelial defense by normal flora (Lu-Walker, 2001).

Two mechanisms of probiotic action have been identified to mediate the maintenance of the gastrointestinal microbial balance: production of antibacterial substances and competitive inhibition of pathogen and toxin adherence to the intestinal epithelium (Vanderpool et al., 2008).

*In vitro* experimental studies have demonstrated that selected lactic acid strains are effective against diarrhoeagenic bacteria. By producing metabolites such as acetic and lactic acids, and thus lowering the pH, a large number of *Lactobacillus* strains inhibit the growth of bacterial pathogens. A strain of *L. lactis* selected for its ability to produce hydrogen peroxide, and *L. casei Shirota* or *L. acidophilus* YIT 0070, reduced the growth of *Escherichia coli* 0157:H7. The cell-free *L. casei* subsp. *rhamnosus* Lcr35 supernatant inhibited the growth of nine human pathogenic bacteria. Lactobacillus strains isolated from the human digestive tract have been found to inhibit the growth of four species known to be anaerobic bacterial aetiological agents of gastroenteric infections (Servin-Coccoiner, 2003).

Probiotic organisms are able to reduce the bacterial load and inflammation of *H. pylori* in animal and human studies. It has been suggested that the suppression effect is strain dependent. *L. casei Shirota* strain showed a significant reduction in the levels of *H. pylori* colonization. *L. johnsonii* La1 and *L. gasseri* OLL2716 were also found to reduce *H. pylori* colonization and inflammation. Similarly, *L. acidophilus* was able to inhibit the growth of *H. pylori*. Regular intake of yoghurt containing *Bifidobacterium animalis* Bb12 and *L. acidophilus* La5 may effectively suppress *Helicobacter pylori* infection.
in humans. Several mechanisms regarding the effect of probiotics on *H. pylori* have been suggested including production of antimicrobial substances, enhanced gut barrier function, and competition for adhesion sites; however, the relative importance of these mechanisms is still unclear (Vasiljevic-Shah, 2008).

It was demonstrated that all bifidobacterial supernatants at pHs between 5.0 and 4.1 were able to produce strain-dependent growth inhibition of clostridia (Trejo et al., 2006).

In the study of Pan et al. (2008), there is characterized the potential probiotic *Lactobacillus acidophilus* NIT originally isolated in infant faeces. Results show that overnight culture of *L. acidophilus* was able to inhibit more pathogens than the supernatant and/or resuspended broth. The overnight culture had a strong inhibition to all the pathogens except *Clostridium histolyticum*. The reduction of *Escherichia coli* and *Salmonella typhimurium* adhesion to Caco-2 cells was more than 50% added with *Lactobacillus acidophilus* $10^8$ CFU per well.

### 3.1 pH-lowering capacity

Probiotic bacteria, especially strains of *Lactobacilli*, produce acetic, lactic, and propionic acid that lower the local pH, leading to inhibiting the growth of a wide range of Gram negative pathogenic bacteria. Some *Lactobacillus* strains inhibit the growth of *Salmonella enterica* solely by the production of lactic acid. However, antibacterial effects of other strains of *Lactobacilli* may be the result of a combination of lactic acid and other unknown *Lactobacillus*-derived bactericidal substances by pH-dependent mechanism (Vanderpool et al., 2008).

The analysis of organic acid composition of bifidobacterial supernatants shows that all strains under study produced lactic and acetic acid with the exception of two strains (539 and NCC 235) in whose supernatants acetic acid was not detected (Trejo et al., 2006).

Short-chain fatty acids (acetic-, propionic-, and butyric acid), which are the fermentation products of saccharides, are repeatedly found in the colon of animals at various concentrations. Their presence in the human colon affects important biological processes, such as growth, metabolism, and differentiation of the intestinal epithelial cells; these processes are vital in maintaining the intestinal barrier integrity. Short-chain fatty acids, in particular butyric acid, affect the intestinal epithelial cell production of inflammatory cytokines that are pivotal to inflammation (Koninkx-Malago, 2008).
3.2 Bacteriocin production

Bacteriocins produced by LAB are classified into three main groups, lantibiotics being the most documented and industrially exploited. The groups are lantibiotics (Class I), nonlantibiotics, small heat-stable peptides (Class II), and large heat-labile protein (Class III) (O’Sullivan et al., 2002).

Studies indicate that these probiotic-derived antibacterial substances exert their effects alone or synergistically to inhibit the growth of pathogens. The 2-component lantibiotics, produced by Gram-positive bacteria, such as Lactococcus lactis, are small antimicrobial peptides. These peptides have been found to be active at nanomolar concentrations to inhibit pathogens by targeting the lipid II component of the bacterial cell wall. Since lantibiotics are ribosomally synthesized and amenable to site-directed mutagenesis, they have the potential to serve as biological templates for the production of novel peptides with improved antibacterial functions. Other non-lanthionine containing bacteriocins are small antimicrobial peptides produced by Lactobacilli (Vanderpool et al., 2008).

Analysis of the known genomic sequences of Lactobacillus strains including L. plantarum, L. acidophilus NCFM, L. johnsonii NCC 533, and L. sakei predicts a broad group of bacteriocins with divergent sequences. These peptides have a relatively narrow spectrum of activity and are mostly toxic to Gram-positive bacteria, including Lactococcus, Streptococcus, Staphylococcus, Listeria, and Mycobacteria. The primary mechanism of bacteriocin action is by forming pores in the cytoplasmic membrane of sensitive bacteria, but they can also interfere with essential enzyme activities in sensitive species. In addition, several strains of Bifidobacteria have been found to produce bacteriocin-like compounds toxic to both Gram-positive and Gram-negative bacteria (Vanderpool et al., 2008).

The lantibiotic nisin naturally produced by Lactococcus lactis ssp. lactis is commercially available as food additive E234. The nisin variants A and Z, differing by one amino acid, are approved for use in foodstuffs by food additive legislating bodies in the US (Food and Drug Administration, FDA) and in the EU (O’Sullivan et al., 2002).

Nisin binds to the carbohydrate moiety of the cell wall precursor lipid II, using it as a docking molecule prior to pore formation.

All forms of nisin are antimicrobially active against Gram-positive bacteria, such as LAB, Listeria sp., Micrococcus sp. and spore-forming bacteria like Bacillus sp. and Clostridium sp. The inhibiting mode of nisin towards vegetative cells consists of several phases. Nisin accumulates on the cell mem-
brane and penetrates into it, then aggregates within the membrane to form a 
water-filled pore (O’Sullivan et al., 2002).

LAB capable of secreting antimicrobial peptides are used in a probiotic 
manner as food preservatives as well as health-promoting agents for humans 
and animals. Nisin applied as a food preservative extends the shelf life of a 
product. It is relatively stable in foodstuffs since 15-20% of nisin is lost in heat 
treatment. For probiotic purposes, bacteriocins are generally produced by a 
LAB strain in the product. The bacteriocin production is highest at the end 
of the exponential and early stationary phase. Some bacterial strains, such 
as Clostridium botulinum 169B and Streptococcus bovis JB1 are resistant to 
nisin. Resistance is assumed to be based on the enzymatic decomposition of 
nisin (Breuer-Radler, 1996).

3.3 Hydrogen peroxide production

The production of hydrogen peroxide (H₂O₂) is a widely accepted hypothesis 
to explain the inhibitory activity of LAB (Charlier et al., 2009).

Servin-Coccoiner (2003) examined a strain of Lactobacillus lactis, selected 
for its ability to produce hydrogen peroxide. L. casei Shirota or L. acidophilus 
YIT 0070 reduced the growth of Escherichia coli 0157:H7. The production 
of hydrogen peroxide by LAB, particularly by lactobacilli, is antagonistic to 
Staphilococcus aureus. The production of hydrogen peroxide by LAB and its 
potential role in S. aureus inhibition is well-documented. Some lactobacilli 
strains are able to inhibit the growth of S. aureus by producing hydrogen 
peroxide at a concentration of 0.18 mmol/l. Hydrogen peroxide has a bacte-
riostatic effect at these concentrations and is bactericidal for concentrations 
up to 0.6 to 1.0 mmol/l. The authors concluded that hydrogen peroxide was 
involved in the capacity of these strains to inhibit S. aureus in mixed culture 
in laboratory media (Vasiljevic-Shah, 2008).

The use of L. delbrueckii ssp. bulgaricus in yoghurt may affect the survival 
of L. acidophilus and Bifidobacterium due to the acid and hydrogen peroxide 
produced during fermentation. The presence of oxygen (positive redox po-
tential) in probiotic-containing products can have a detrimental effect on the 
viability of probiotics. Strains of L. acidophilus and Bifidobacterium spp. are 
microaerophilic and anaerobic, respectively. They lack an electron-transport 
chain, which results in the incomplete reduction of oxygen to hydrogen per-
oxide. Furthermore, they are devoid of catalase, thus incapable of converting 
hydrogen peroxide into water, the intracellular accumulation of hydrogen per-
oxide thus causing death of the cell was observed (Vasiljevic-Shah, 2008).
3.4 Competition at adhesion sites

Part of the beneficial effect of probiotics is reducing the establishment of pathogenic bacteria, such as *Salmonella*, a phenomenon called competitive exclusion. Probiotic bacteria interfere with the pathogen-receptor or toxin-receptor interactions. Preincubation of polarized monolayers of fully differentiated, villus-like Caco-2 cells with *Lactobacillus plantarum* MF1298 attenuated a decrease in transepithelial electrical resistance induced by *Listeria monocytogenes* (*Konink-Malago*, 2008).

Probiotics in the gastrointestinal tract decrease adhesion of both pathogens and their toxins to the intestinal epithelium. Several strains of *Lactobacilli* and *Bifidobacteria* are able to compete with pathogenic bacteria, including *Bacteroides vulgatus*, *Clostridium histolyticum*, *C. difficile*, *Enterobacter aerogenes*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella enterica*, *Yersinia enterocolitica*, enterotoxigenic *E. coli*, and enteropathogenic *E. coli* for intestinal epithelial cell binding, and they can displace pathogenic bacteria even if the pathogens have attached to intestinal epithelial cells prior to probiotic treatment. However, specific probiotics or probiotic combinations should be selected based on their ability to inhibit or displace a specific pathogen (*Vanderpool et al.*, 2008).

Live and heat-killed *L. acidophilus* strain LB, which adheres to Caco-2 cells, inhibits adhesion to the brush border of diarrhoeagenic ETEC bearing colonization factor antigen CFA I or CFA II adhesive factors, in a concentration-dependent manner. Live and heat-killed *L. acidophilus* strain LB inhibits both cell association and the invasion of Caco-2 cells by enterovirulent *S. enterica* serovar *typhimurium*, EPEC, *Ersonia pseudotuberculosis* and *Listeria monocytogenes* in a concentration-dependent manner. Incubating Caco-2 cells with La1 was more effective before and during infection with enterovirulent *E. coli* than after infection by *E. coli*, indicating that La1 was able to prevent cell infection (*Servin-Coccoiner*, 2003).

Blockade of bacterial enterotoxin binding has also been demonstrated as a mechanism with therapeutic potential. The virulence factor of enterotoxigenic *E. coli* strains is a heat-labile enterotoxin that induces traveller’s diarrhoea by binding to ganglioside GM1 on the surface of intestinal epithelial cells. By using a toxin-receptor blockade strategy, an engineered probiotic bacterium was generated to express glycosyltransferase genes from *Neisseria meningitides* or *Campylobacter jejuni* in a nonpathogenic *E. coli* strain (CWG308) (*Vanderpool et al.*, 2008).
Preincubation of *C. difficile* 43593 with neutralized supernatant of bifidobacterial strain CIDCA 5320 and CIDCA 5323 produced a decrease in the adhesion of clostridia to enterocytes (Trejo et al., 2006).

The possible effect of *Lactobacillus gasseri* K7 strain to inhibit the adhesion of *Escherichia coli* O8:K88 to intestinal mucosa was studied by two models: on cultured Caco-2 cells and on small intestinal tissue of pigs. Lactobacilli were added simultaneously with *E. coli* (for competition assay), or the addition of lactobacilli alone was followed by the washing of unbound cells and the addition of *E. coli* cells (for exclusion assay). Preventive inoculation with K7 strain decreased the severity of infection and protected piglets against perishing, although it did not totally protect them against infection. Only the piglets inoculated with K7 strain did not show any infection symptoms and were in very good condition, which indicates the safe use of K7 strain. The applied lactobacilli probably prevented the adhesion of *E. coli* by competitive exclusion, which can include the occupation of specific *E. coli* binding sites or non-specific sterical hindrance of *E. coli* binding by lactobacilli (Rogelj-Matijasic, 2006).

In the study of Candela et al. (2008), strains belonging to *Bifidobacterium* and *Lactobacillus* were screened for their capability to compete with enteropathogens for enterocyte adhesion. The cell lines Caco-2 and HT29 were employed in adhesion experiments. It was demonstrated that *B. longum* Bar33, *B. lactis* Bar30, *L. acidophilus* Bar13, and *L. plantarum* Bar10 are effective in inhibiting the adhesion of *S. cholerasuis serovar typhimurium* and *E. coli* ETEC to Caco-2 cells. *L. acidophilus* Bar13 and *B. longum* Bar33 have the potential to protect intestinal cells from an acute inflammatory response.

Antagonistic effects of isolated lactic acid bacteria in our experiments were certificated using intestinal pathogen bacteria, which frequently cause food poisoning or intestinal diseases. Our isolated strains – exactly seven strains – produced metabolites, which have bacteriostatic effect on *Salmonella enteritidis* (Both et al., 2009).

### 4 Stimulation of the immune system

The intestinal mucous membrane plays an important role in the exclusion and elimination of potentially harmful antigens and microorganisms and simultaneously provides the selective absorption of nutrients (Herich-Levkut, 2002).

The discovery that probiotics can stimulate an immune response provides a scientific basis for some of the observed probiotic effects. It was demon-
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Stratified that administration of *L. casei* induced an increase in the production of circulating antibodies against *Pseudomonas aeruginosa*. Previous studies encouraged the use of certain lactobacilli as immunopotentiators. For example:

- it is important to know the type of immune cells that the LAB are able to stimulate, to know whether the induced immune response will be beneficial or not for the host (inflammatory or specific immune response);
- which is the most active strain;
- the dose required for maximum effect;
- when should it be administered;
- is it safe to use LAB or fermented milks in an immunosuppressed host (*Perdigon et al.*, 2001).

As mentioned above, probiotics serve not only to stabilize the gut microbiota but they can also potentially modulate the function of immune cells. Microorganisms in the gut lumen are recognized and processed by the immune system through several routes. a) Specialized epithelial cells called M (microfold) cells in the follicle-associated epithelium covering Peyer’s patches or in the villi can take up probiotics directly by transcytosis. Macrophages (Mfs) or dendritic cells (DCs) are present immediately below M cells and then engulf probiotics and trigger immune responses. b) DCs in the intestinal lamina propria have been found to extend their dendrites between intestinal epithelial cells (IECs) and might directly sample and process probiotics in the gut lumen. c) Probiotics directly affect IECs to secrete an array of cytokines, which in turn modulate the immune functions of DCs, T cells, and B cells in the gut-associated lymphoid tissue (GALT) (*Shida-Nanno*, 2008).

Macrophages and DCs exposed to probiotics can be observed to secrete a variety of cytokines. Chief among these are IL-12 and IL-10, which are key to controlling the balance of the immune response because the former augments cellular immunity whereas the latter suppresses inflammatory responses. Comparative analyses have revealed that the abilities of *Lactobacillus* strains to induce IL-12 production by macrophages are highly variable. Dietary supplementation with *L. rhamnosus* HN001 has been shown to increase NK cell number in humans and *Lactobacillus casei* Shirota-fermented milk enhances cytotoxic activity of NK cells (*Shida-Nanno*, 2008).
Figure 2: Three hypothetical pathways by which probiotics can trigger and modulate immune function in the intestine (Shida-Nanno, 2008)

There is accumulating evidence showing that dietary supplementation with probiotics augments innate immune functions including phagocytic activity of neutrophils and cytotoxic activity of NK cells. The activation of neutrophils and NK cells might be closely connected with the anti-infectious or anticancer abilities of probiotics. The abilities of lactobacilli to elicit IL-10 production from human DCs and PBMCs are weaker than those of bifidobacteria. That said, the addition of some *Lactobacillus* strains at high doses could induce high levels of IL-10, resulting in a decrease of IL-12 production in murine macrophage or DC cultures (Shida-Nanno, 2008).

Probiotics might also exert their anti-inflammatory activity by inhibiting the secretion of inflammatory cytokines such as IL-6 and IL-8. Furthermore, live but not heat-killed *L. casei* DN-114 001 cells could effectively downregulate spontaneous secretion of TNF-α by the inflamed mucosa of CD patients. Similarly, Caco-2 cells pretreated with live and heat-killed *L. rhamnosus* GG secreted much lower levels of IL-8 after stimulation with TNF-α (Shida-Nanno, 2008).
There are considerable differences in the ability of different probiotic bacteria to induce IL-12 and IFN-g. *Lactobacillus* and *Bifidobacterium* strains, which have previously been shown to stimulate IL-12 and IFN-g production in human PBMC, in the present study, were found to be relatively poor inducers of these cytokines. *S. thermophilus* and *Leuconostoc* strains used in the present study were extremely potent inducers of IL-12 and IFN-g, IL-10 was induced by *Bifidobacterium* and *Propionibacterium* strains whereas IL-10 production induced by *Streptococcus*, *Lactobacillus*, *Lactococcus*, and *Leuconostoc* strains remained at a low level (Kekkonen et al., 2008).

There is a clear difference between Gram-negative non-pathogenic bacteria and LAB in their interaction with IEC. In direct interaction with IEC, both types of bacteria induce IFN-γ but the stimulating effect of LAB is restricted to the cellular surface molecule expression (Herich-Levkut, 2002).

Recently, IL-17-producing T helper cells (Th17 cells) have been regarded as crucial for the pathogenesis of several chronic inflammatory diseases. Th17 cells are abundant in the intestine; orally administered probiotics could affect the development of Th17 cells and ameliorate clinical symptoms. Regulation of Th17 cell functions might be the next target for probiotic modulation of the mucosal immune system (Shida-Nanno, 2008).

In sum, probiotics could potentially play a role in the control of the entire immune network by affecting diverse sets of immune-regulatory cells in the intestine.

The suppression of the formation of pro-inflammatory cytokines and chemokines in the presence of probiotics has been reported in several in vitro studies. The response of the immune system to a probiotic was weaker than in the presence of a Gram-positive pathogen. The cytokine response may vary greatly in the presence of different probiotics. The mixture of eight different probiotic and LAB strains including *L. acidophilus*, *L. delbrueckii ssp. bulgaricus*, *L. casei*, *L. plantarum*, *B. longum*, *B. infantis*, *B. breve*, and *S. thermophilus* upregulated the production of IL-10 (Vasiljevic-Shah, 2008).

In vitro immunomodulating capacity and mechanisms of LAB isolated from kefir grains and their individual supernatants by cytokine profiles were examined in the study of Hong et al. (2009). Time-dependent increases in cytokine levels were observed for both TNF-a and IL-6. There was no secretion of IL-12 and IL-1b for all treatments. The secretion of TNF-a, induced by isolated and reference strains – except for reference strain *Lb. kefiranofaciens* – was not changed compared to the kefir supernatant control whereas the production of IL-1b and IL-12 was significantly decreased. For IL-6, except for *Lb. kefiranofaciens* M1, low or no secretion was observed after co-cultured with isolated
strains. The secretion of IL-10, induced by isolated and reference strains – except for reference strain *Lb. kefiranofaciens* – was higher compared to the kefir supernatant control.

*Bifidobacterium* and *Lactobacillus* strains were assessed for their immuno-modulating activity in the study of Candela *et al.* (2008). The cell lines Caco-2 and HT29 were employed in immune response experiments. The enterocyte-like Caco-2 cells have been extensively used to investigate the adhesion of probiotic bacteria to enterocyte. The enterocyte-like HT-29 cells represent a well-characterized model to study the enterocyte immune response to bacterial infection. Results show that neither probiotic strain induces the IL-8 secretion.

**References**


