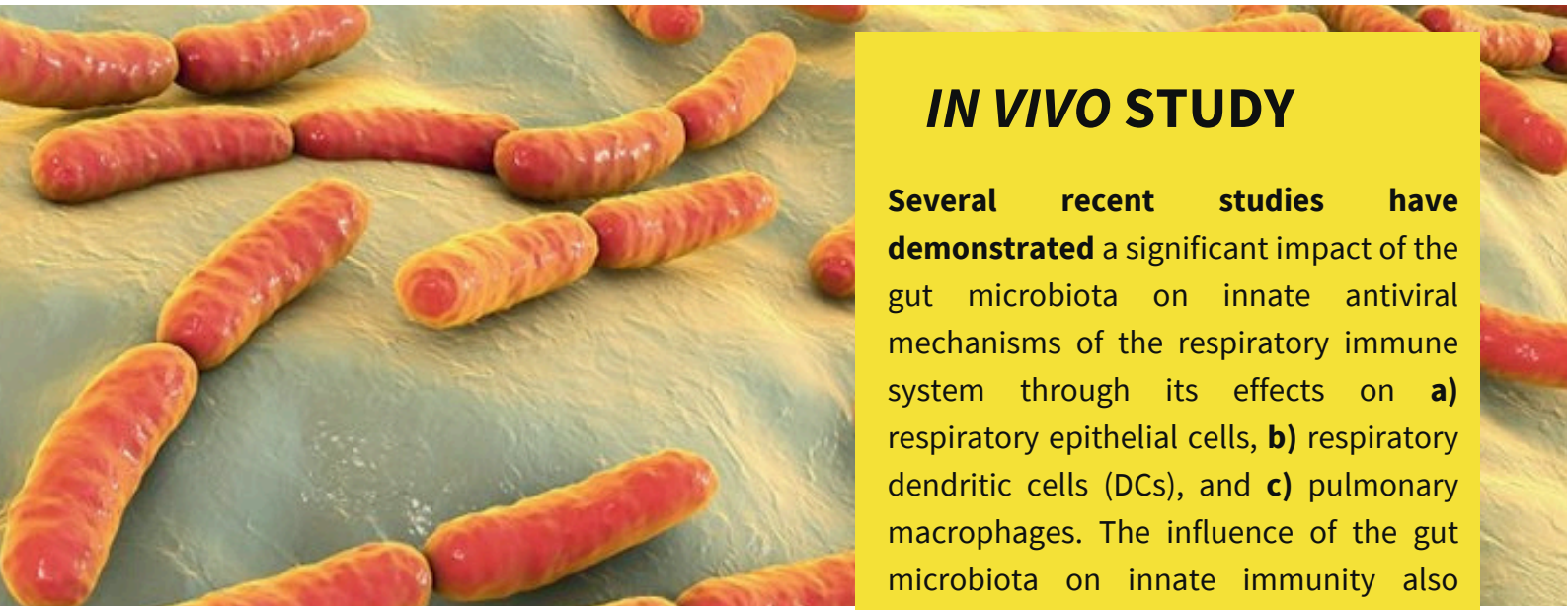
# Modulation of Human Respiratory Immunity with Immunobiotics

**Targeted combination of the beneficial bacterium *Lacticaseibacillus rhamnosus* CRL1505, beta-glucans, and vitamin D**



## A targeted combination of the beneficial bacterium *Lacticaseibacillus rhamnosus* CRL1505, beta-glucans, and vitamin D

The combination of the lactic acid bacterium *Lacticaseibacillus rhamnosus* CRL1505 (Lr1505) and beta-glucans was selected based on their scientifically and clinically proven ability to modulate immune responses outside the gastrointestinal tract, including the respiratory mucosa.


## *IN VIVO* STUDY

**Several recent studies have demonstrated** a significant impact of the gut microbiota on innate antiviral mechanisms of the respiratory immune system through its effects on **a)** respiratory epithelial cells, **b)** respiratory dendritic cells (DCs), and **c)** pulmonary macrophages. The influence of the gut microbiota on innate immunity also modulates humoral adaptive immune responses (Figure 1) (1).

To study the mechanisms involved in enhancing respiratory antiviral immunity mediated by Lr1505, animal model studies were conducted using two respiratory viruses: respiratory syncytial virus (RSV) and influenza virus (IFV) (2,3).

To investigate the mechanisms involved in enhancing both gut and respiratory antibacterial immunity mediated by Lr1505, animal studies were performed using the bacteria Salmonella enterica serovar Typhimurium and Streptococcus pneumoniae (4).

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**1) Respiratory Innate Antiviral Immune Response and L. rhamnosus CRL1505**

The study results showed that orally administered Lr1505 was able to significantly reduce viral titers in the lungs, decrease lung damage, and increase survival in mice in response to RSV (2) or IFV (3) infections. The protective effect induced by Lr1505 was associated with its ability to increase type I IFN levels in the respiratory tract (1,2,3).

Lr1505 was also shown to enhance antigen presentation, which could have a beneficial effect on the respiratory humoral immune response (1).

**3) Intestinal Antiviral Immune Response and L. rhamnosus CRL1505**

Further studies conducted on intestinal epithelial cells showed that Lr1505 is capable of increasing

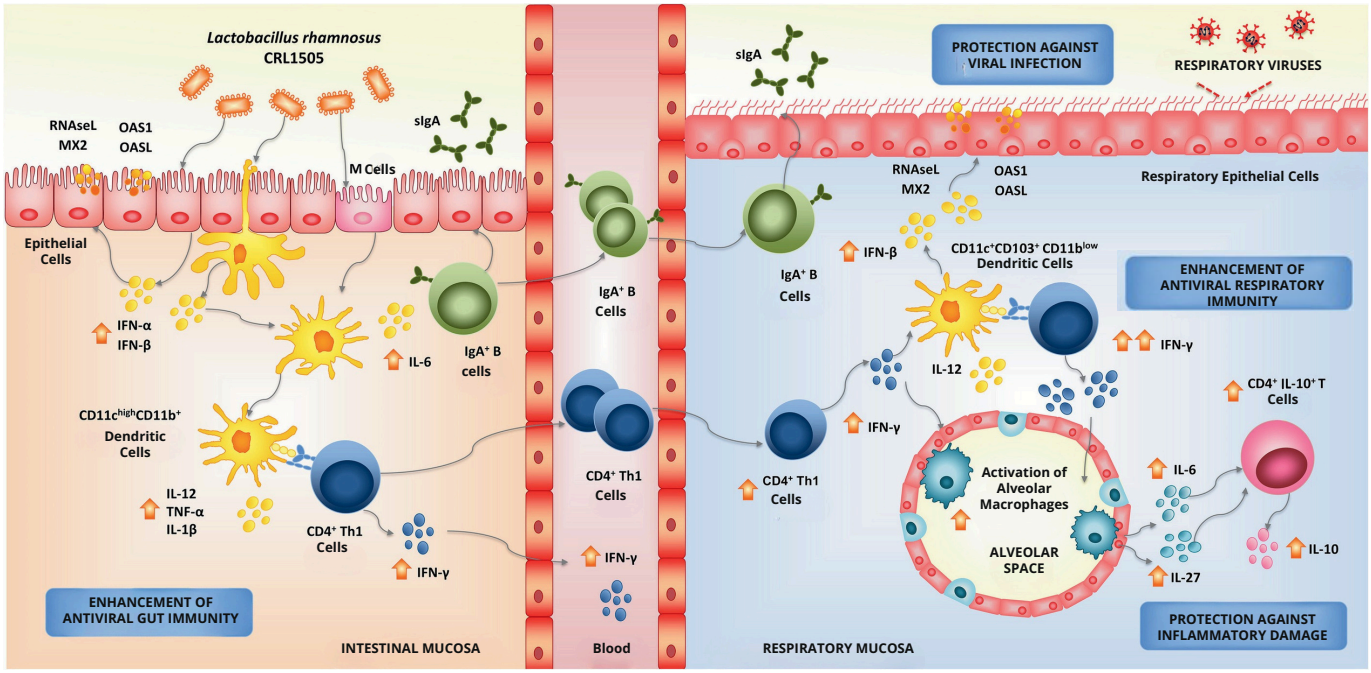


**Figure 1.** Modulation of respiratory antiviral immunity by *Lacticaseibacillus rhamnosus* CRL1505. Proposed mechanism for distal immunomodulation induced by the immunobiotic strain *L. rhamnosus* CRL1505 and enhanced resistance to viral infections through the improvement of respiratory innate and adaptive antiviral immune responses. (Adapted from Villena and Kitazawa, 2020)

The probiotic strain Lr1505 induced the mobilization of CD4(+)IFN-γ(+) T cells from the gut to the respiratory tract, thereby increasing local IFN-γ production—a key activator of both innate and adaptive immunity—and stimulating antigen-presenting cells (2,5) (Figure 1).

**2)Respiratory Adaptive Antiviral Immune Response and L. rhamnosus CRL1505**

Evaluation of the pulmonary dendritic cell (DC) population in mice after oral administration of Lr1505 showed increased levels of CD11c(+)CD103(+) and CD11c(+)CD11b(high) DCs.

type I IFN production (6), enhance the expression of IFN-α and IFN-β, as well as various antiviral factors (Nlrp3, Oas1, etc.) required for effective defense against viruses (7).

**4) Antibacterial Immune Response and L. rhamnosus CRL1505**

Oral supplementation with Lr1505 was also effective against enteropathogenic bacteria and was shown to stimulate the respiratory mucosal immune system. By increasing IFN-γ levels in serum and intestinal fluid, Lr1505 activated peritoneal macrophages, leading to enhanced elimination of the enteropathogen S. *typhimurium*.

***(Salmonella enterica serovar Typhimurium) (8).***

Administration of Lr1505 also significantly increased levels of antipneumococcal respiratory and serum IgA and IgG antibodies, indicating a beneficial effect on the antibody response during ***Streptococcus pneumoniae infection (4).***

## CLINICAL STUDIES

- 6-month (July–December), on a cohort of 298 children with Lr1505
- The mechanism of immunomodulation also demonstrated in an in vivo and clinical 8-week study, on a cohort of 60 participants with heat-killed Lr1505
- (HkLr1505)
- Randomized
- Double-blind
  Placebo-controlled

**The results of the first study**, conducted in a group of children aged 2–5 years, suggest a significant reduction in the severity and incidence of gastrointestinal and respiratory infections, especially in young children, through the direct antimicrobial effect and enhancement of mucosal barrier function due to the effects of Lr1505 on both innate and adaptive immunity.

**The results of the mechanistic study**, conducted on a population of 60 healthy adults, also highlighted the ability of heat-killed Lr1505 to maintain an optimal immune state and physical condition by acting on and activating pDC cells, which influence overall immune function, confirming the immunomodulatory potential of the live Lr1505 strain.

## Introduction

**Infectious diseases** such as acute otitis media, pharyngitis and tonsillitis, upper respiratory tract infections, pneumonia, bronchitis, and diarrhea remain among the leading causes of death in preschool-aged children (9–11).

**The rise of antibiotic resistance** and the need for new and improved strategies to address infectious diseases have led to the exploration of **the therapeutic potential of induced modulation of mucosal immune responses using probiotics.**

As a result, it has been found that certain lactic acid bacteria (LAB) probiotics have protective effects against bacterial and viral infections in the gastrointestinal tract (12).

Significant attention has been focused on the role of probiotics in protecting the gut from pathogens. However, growing evidence indicates that orally administered probiotics are also capable of regulating immune responses beyond the gastrointestinal tract, including the respiratory mucosa (13).

The first clinical study revealed that the probiotic Lr1505, added to yogurt, **enhanced and strengthened mucosal immunity and reduced the incidence and severity of intestinal and respiratory infections in children (14).**

**In 2023, clinical studies using a dietary supplement in both pediatric and adult populations were completed, and the results correspond with the findings from the probiotic yogurt study. This indicates that the delivery vehicle for the bacteria is not crucial, and their effect is transferable. The studies are expected to be published during 2024.**

## Methodology

Healthy children attending daycare centers five days a week were enrolled in the study. The probiotic strain Lr1505 was added to sweetened yogurt at a concentration of at least 1×10^8 colony-forming units (CFU) per 100 g (14).

The placebo group received the same yogurt without Lr1505.

Both groups were monitored throughout the 6-month study by nutritionists, pediatricians, and immunologists.

At the beginning and end of the study, stool samples were collected to determine the number of lactic acid bacteria. In addition, salivary IgA levels were measured (14).

# Results

## 1) Incidence of Infections

Out of the total 298 children who participated in the study, 132 (45%) experienced an infectious disease during the study. The most common **infections were upper respiratory tract infections, followed by pharyngitis and tonsillitis, and diarrhea.**

In the group of children receiving yogurt with the probiotic strain L. rhamnosus CRL1505, a significant **reduction in infectious diseases** was observed compared to the placebo group (34% vs. 66%). Further analysis revealed significant differences between the placebo group and the Lr1505 group in the incidence of specific infections: upper respiratory tract infections (31% vs. 69%) (**Figure 2A**), pharyngitis and tonsillitis (28% vs. 72%) (**Figure 2B**), and acute diarrhea (26% vs. 74%) (**Figure 2C**) (14).

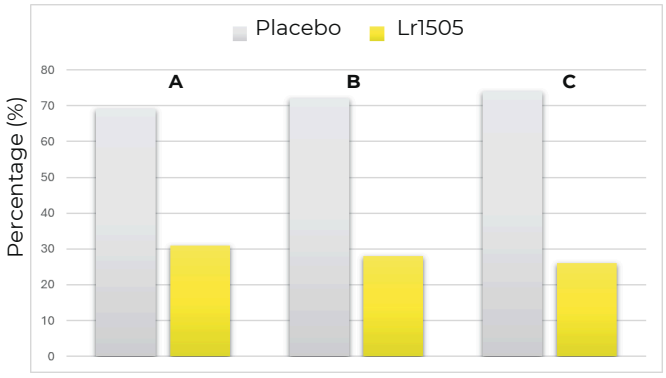


**Figure 2:** Comparison of the incidence of infectious diseases in the study groups. **A:** Upper respiratory tract infections **B:** Tonsillitis and pharyngitis **C:** Diarrhea **(**Adapted from Villena et al., 2012)

The clinical effects of probiotic supplementation on the incidence of fever and the need for antibiotic treatment were also evaluated. It was found that subjects in the Lr1505 yogurt group had a significantly lower incidence of fever (**Figure 3A**). In addition, **the need for antibiotic treatment** among children consuming probiotics was significantly lower than in the placebo group (**Figure 3B**) (14).

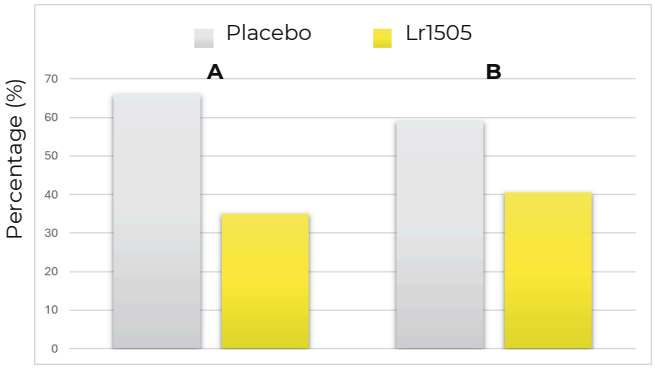


**Figure 3:** Percentage comparison of **A)** presence of fever and **B)** need for antibiotic therapy in the study groups (Adapted from Villena et al., 2012)

## 2) Salivary Immunoglobulin A (IgA) Levels

Salivary IgA was also measured to assess **mucosal immunity.** Samples were collected one day before the start of the intervention (baseline levels) and at the end of probiotic or placebo administration (post-supplementation levels). Comparison between the probiotic yogurt and placebo groups showed no significant differences in baseline IgA levels.

In contrast, **a significant increase in IgA levels was observed in children receiving the probiotic yogur**t, while IgA levels in the placebo group remained similar to those measured at the beginning of the study. Consumption of probiotics containing the Lr1505 strain is therefore capable of enhancing mucosal immunity, as evidenced by salivary IgA levels (Figure 4) (14).

**The study results** suggest that the Lr1505 probiotic helps reduce the incidence of upper respiratory and gastrointestinal infections, supports the immune system, and decreases the need for antibiotic use.

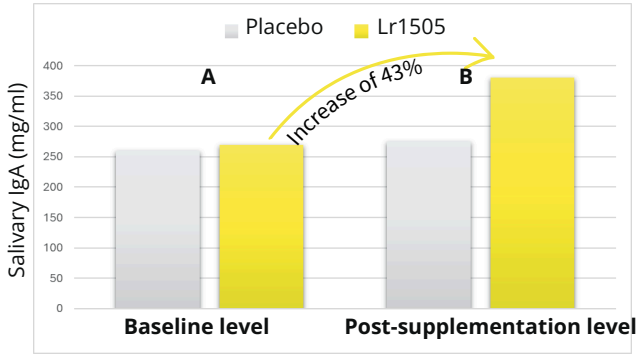


**Figure 4.** Salivary IgA levels in the Lr1505 and placebo groups. (Adapted from Villena et al., 2012)

# MECHANISM OF ACTION

The human immune system represents an effective defense mechanism against the omnipresent bacteria and viruses we are exposed to every day, and its vitality needs to be maintained and supported.
It is assumed that **plasmacytoid dendritic cells (pDCs)** play an important role in the activation and regulation of immune functions. Activation of pDCs stimulates various immune cells responsible for innate and adaptive immunity by increasing IF-α, HLA-DR, and other key immune molecules, which contributes to the normal maintenance of overall immune function (16).

This clinical study also revealed the potential of **heat-killed Lactobacillus rhamnosus 1505 (HkLr1505)** to support **the maintenance of normal immune function through the activation of pDCs**, as well as to reduce disease symptoms during infection (15), reinforcing the findings of a previous clinical study (14).

## Methodology

The clinical study was conducted on healthy individuals of both sexes, aged 20–64 years, over a period of 8 weeks. Participants had no history of serious or chronic illness, were not undergoing any medical treatment, had never suffered from intestinal diseases affecting digestion or absorption, and had not experienced any respiratory tract infections within the past two years. Participants were administered tablets containing either (**a**) HkLr1505 or (**b**) no HkLr1505 (placebo group) (15).

pDC activity was evaluated by measuring the expression levels of HLA-DR and CD86. The overall health status was assessed through a series of health questionnaires and monitored by a physician (15).

## Results

**1) HLA-DR Levels (%)**

When assessing immune function, HLA-DR levels decreased in both groups after 8 weeks of supplementation but remained significantly higher in the HkLr1505 group compared to the placebo group (15).

**2) Overall Physical Condition**

When evaluating physical condition, the cumulative number of days with partial symptoms (such as nasal congestion, throat discomfort, chest heaviness, hoarseness, headache, nausea, joint and muscle pain, and stomach pain during the intake period) was significantly reduced in the HkLr1505 group compared to the placebo group (15).

**3) PG05 – A Key Aspect of the Immunomodulatory Activity of Lactobacillus rhamnosus 1505**

One of the key elements identified in the interaction between Lr1505 or HkLr1505 and the host is its **strain-specific peptidoglycan** (PG05; a component of the cell wall of both live and heat-killed bacteria), which has been shown to possess **immunomodulatory activity**. PG05 significantly enhances the production of specific antibodies and increases the number of alveolar macrophages (AM) producing IFN-β. These cells play a crucial role in the beneficial modulation of the innate immune response during **viral** and **bacterial infections** (17).

## Conclusion

The conclusions of the study investigating the mechanism of action behind the immunomodulatory activity of heat-killed Lactobacillus rhamnosus 1505 confirm and strengthen the findings of previous clinical research conducted in a pediatric population, as well as those from a clinical study carried out in an adult population, which is expected to be published during 2024.

# IMMUNOMODULATORY EFFECT OF BETAGLUCANS

Currently, most prebiotics on the market are derived from indigestible oligosaccharides. However, recent research has also focused on indigestible long-chain complex carbohydrates, such as **beta-glucans**, obtained from various food sources. These have demonstrated not only health-promoting effects but also the potential as a novel source of prebiotics. Recent studies have investigated the impact of consuming long-chain **(1,3)-(1,6)-β-D-glucan** isolated from **the yeast *Saccharomyces cerevisiae*** on the number of cold episodes in healthy individuals through a placebo-controlled, double-blind, randomized, multicenter clinical trial, which demonstrated an enhanced ability of the human immune system to defend against invasive pathogens (18).

Long-chain (1,3)-(1,6)-β-D-glucan from *Saccharomyces cerevisiae* stimulates **immune *cells in* the gut to initiate either a nonspecific or specific immune response.** It activates "scavenger" cells in the so-called Peyer's patches (macrophages), which begin by destroying pathogens and subsequently activate other defense cells, such as B and T lymphocytes or dendritic cells. By supporting white blood cells, this beta-glucan contributes significantly to the body's defense against bacteria, viruses, fungi, and parasites that cause disease (19).

## Vitamin D

Enzymes that metabolize vitamin D and vitamin D receptors are also present in immune cells, including antigen-presenting cells, T cells, B cells, and monocytes. In vitro data show that, in addition to modulating innate immune cells, vitamin D promotes a more tolerogenic immune state. In vivo data from animal studies and human vitamin D supplementation studies have demonstrated beneficial effects of vitamin D on immune function, particularly in the context of autoimmunity (20).

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