For Immunostimulation, counteracting allergies, beauty effects

# IMMUNOL®

IMMUNOL® is made by fermenting plant-derived raw materials with acetic acid bacteria, a bacteria isolated from the persimmon fruit.

## Immune balance - activating innate immunity -

● Lipopolysaccharide (LPS), an active ingredient of IMMUNOL®, is a cell wall component of Gram-negative bacteria such as acetic acid bacteria, and is expected to balance body immunity and enhance spontaneous recovery and skin metabolism.

●Ingesting IMMUNOL activates macrophages, a phagocyte.

●Taking it with lactobacilli is expected to activate macrophage further with its synergistic effects.

●Ingestion of IMMUNOL activates mucosal immune function and can be expected to prevent infectious diseases from invading foreign enemies.

## Immune balance - improving allergic predisposition -

IMMUNOL is effective in suppressing IgE antibody-dependent allergies (type I allergy), especially with symptoms of pollinosis and atopic dermatitis.

## Activating fibroblasts - good for beauty -

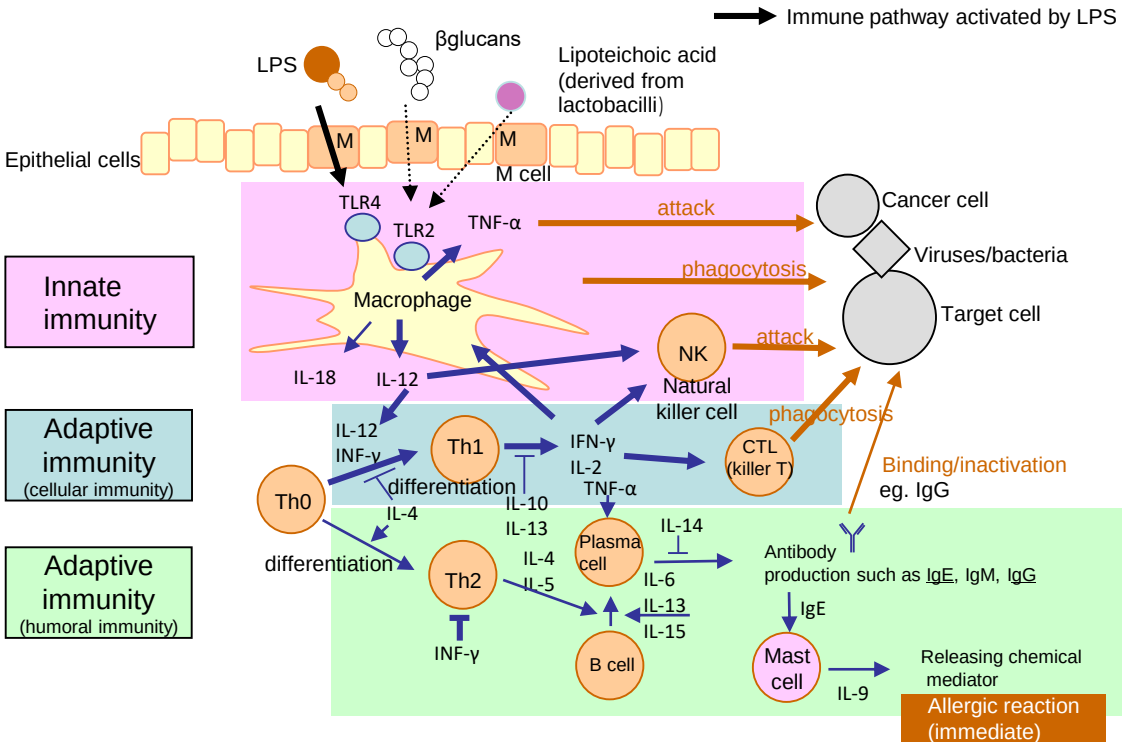
IMMUNOL promotes the proliferation and hyaluronic acid production in fibroblasts.

| Product name | IMMUNOL (Fermented product of acetic acid bacteria) |
|---|---|
| Name | Processed acetic acid bacteria-fermented product |
| Example of description of raw materials | Acetic acid bacterial fermentation product ,Dextrin |
| Standard amount to be used | 50 – 100 mg/day |
| Package | 1 kg/aluminum bag |
| Storage | Store it away from direct sunlight and high temperature/humidity |
| Expiration date | 3 years from production date |

| TEST | SPECIFICATION | TEST METHOD |
|---|---|---|
| Appearance | Light yellow or yellowish brown powder | Visual |
| Foreign materials | Not detectable | Visual |
| LPS (Lipopolysaccharide) | More than 60μg/g | Limulus |
| Moisture | Not more than 7% | Normal pressure heating and drying method |
| Heavy metal (as Pb) | Not more than 20ppm | Sodium sulfide colorimetry |
| Arsenic (as $As_2O_3$) | Not more than 2.0ppm | ICP |
| Aerobic plate count | Not more than 1000cfu/g | Pour plate culture |
| Coliforms | Negative | BGLB |
| Viable molds and yeasts count | Not more than 100cfu/g | AOAC |

## Mechanism of Immunostimulation by LPS

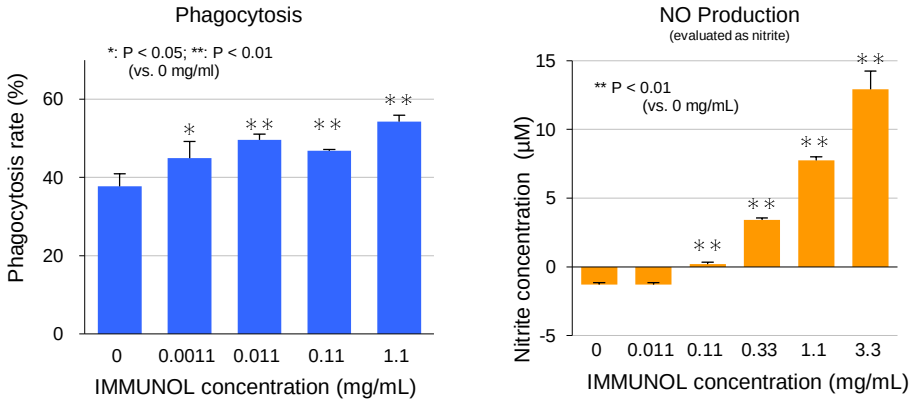
Lipopolysaccharide (LPS) contained in IMMUNOL is recognized by a complex Toll-like receptor (TLR) 4 located on the surface of the cells, such as macrophages. Then, transcription factors such as NF$\kappa$B or IRF, are activated through various signaling and imported into the nucleus to introduce expression of genes of cytokines, which play important roles in the immune system, such as TNF$\alpha$ or interleukins (ILs). Well known immunostimulators such as $\beta$glucans, peptideglycans, lactobacilli and others, are signaled similarly via TLR2. NO production, which is an indicator of macrophage activation, is 1000 to 10000 times higher than $\beta$glucan, and LPS efficiently introduces immunostimulation activation. Additionally, LPS stimulates cellular immunity mainly by enhancing the innate immunity and Th1, but not Th2, resulting in the reduction of allergic reactions.



TOYO HAKKO

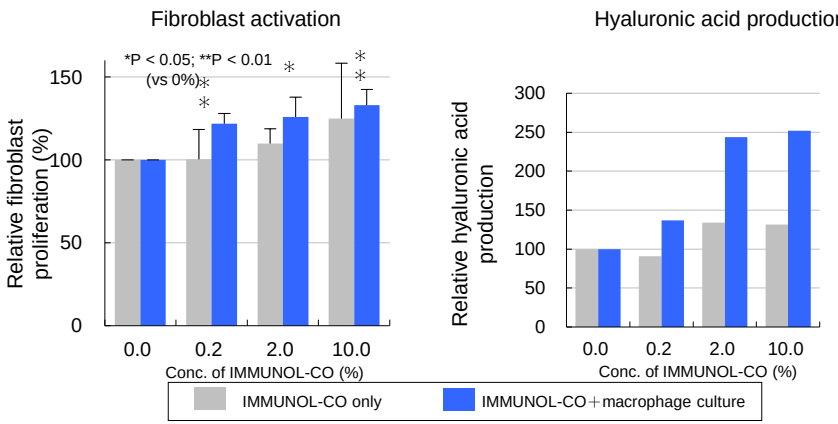
## Testing to evaluate the Innate Immunity Activation (Macrophage activation)

<Tests and Methods>
In the cultures of the macrophage cell lines (J774.1, RAW264.7) with various concentrations, IMMUNOL was added and incubated to achieve the concentrations indicated in the Figures.
After adding PE-labelled polylatexbeads and recovering the cells, phagocytosis was evaluated by flow cytometry.
NO production was evaluated by adding the Griess reagent to the cell supernatant and incubating at room temperature, then measuring OD at 550 nm/668 nm and calculating the nitrite concentration.
<Results>
By adding IMMUNOL, increases in Phagocytosis and NO production were determined to be concentration dependent.




## Beautiful skin effects by activating innate immunity (activating macrophages)

<Tests and Methods>
After IMMUNOL-CO was added to macrophages, a type of immune cells, the cells were cultured for 24 hours. The culture was added on fibroblasts and the cells were incubated to determine the fibroblast proliferation percentage and hyaluronic acid production. As a comparison, fibroblasts without adding the culture of the stimulated macrophages but just IMMUNOL-CO was also determined.
<Results>
The fibroblast proliferation and hyaluronic acid production were promoted even when just IMMUNOL-CO was added (without macrophage stimulation); those actions were determined to be further increased by activating macrophages, a type of immune cells. From the results, IMMUNOL-CO is expected to have beautiful skin effects by promoting skin turnover through immunostimulation.

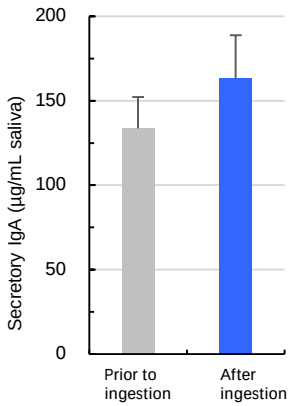



## Activate the immune function in humans

<Test method>
An immunopotentiative action evaluation test was conducted on 12 healthy adults (8 males and 4 females, ages 29-62). Each test subject was given 1 chewable tablet containing 100 mg of IMMUNOL per day for a period of 28 days. Secretory IgA, NK cell activation, and IFN-γ (interferon-γ) were evaluated as factors related to the mucosal immune function.
<Results>
Ingesting 100 mg of IMMUNOL increased secretory IgA, NK cell activation, and IFN-γ. Given this result, IMMUNOL can be expected to be used as a material for controlling infection and preventing viruses and bacteria from invading the body by activating the mucosal immune function.

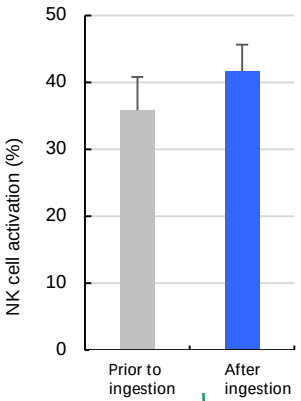
**Secretory IgA**

Secretory IgA binds to pathogens, neutralizes the toxicity, and inhibits adhesion to the mucous membrane, thereby preventing invasion into the body.




**NK cells**

As soon as an NK cell finds a cell infected with a virus or cancer cell, it attacks and completely destroys it.




**IFN-γ**

Produced by helper T cells, NK cells, and other immune system cells, it activates macrophages and NK cells, thereby increasing the immune response.