

# Immune effect of lipopolysaccharide (LPS) supplement -Treatment of parvovirus infection and atopic dermatitis-

Hiroyuki INAGAWA<sup>1)</sup>, Hiroshi OKAWA<sup>2)</sup>, Akihisa KATO<sup>3)</sup>

<sup>1)</sup>Integrated Immune System Course, Faculty of Medicine, Kagawa University, Kagawa, Japan;

<sup>2)</sup> Scarecrow Incorporated, Tokyo, Japan; <sup>3)</sup> D&C Veterinary Clinic, Ibaraki, Japan

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Natural immunity is thought to be at the center of the individual maintenance mechanism, a key factor of self-curing power. To put it another way around, conditions in which natural immunity cannot work normally trigger the development of lifestyle diseases. We have focused on the usefulness of natural immunity in health maintenance, and developed various uses of this system for disease prevention and treatment. Through our studies, it has been clarified that the LPS of *Pantoea agglomerans*, plant-symbiotic gram-negative bacteria having abundant meal experiences, shows anti-infective and antiallergy effects in human and mouse studies.

Allergic disorders are caused by immune system's being out of balance, which triggers an overactive immune response to the substances normally present in the natural environment. Many virus infections are developed by viruses incorporated in the cell via receptors, which then cause cell apoptosis to induce inflammation. In both cases, natural immunity is involved in the vital control at the initial stage.

In this seminar, we propose a new aspect of natural immunity and present our investigation results on plant-symbiotic *Pantoea agglomerans* LPS, a new natural immunity-regulating material, especially on its improvement effect on canine allergic diseases and canine parvovirus disease (CPVD), which still has no established way to deal with. We assessed the antiallergic effect of LPS in dogs, and the efficacy rate reached 58.8% in 153 cases. Then we assessed its effect on CPVD by survival rate. When LPS was administered prophylactically before onset of CPVS, the survival rate was 85.7%. It was statistically significantly higher than the survival rate of LPS-free group, 55.6%.

The findings mentioned above show that orally administered LPS exhibits a useful effect in disease prevention as a natural immunity-regulating material. Since LPS has almost no adverse reactions, development of its new usage is thought to be promising if it is used from the perspective of enhancing drug effectiveness.

**Keywords:** LPS, canine parvovirus (CPV) infection, *Pantoea agglomerans*, atopic dermatitis.

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## INTRODUCTION

The essence of natural immune function is identification and elimination of foreign bodies, a role played mainly by macrophages. These days, many diseases including lifestyle diseases, allergic disor-

ders, and cancers are thought to be caused by chronic inflammation induced by foreign bodies produced by the body such as oxidized LDL, AGEs (advanced glycation end products), and disordered proteins. Macrophages remove those foreign bodies from the inside of blood vessels and tissues by ingesting them

via recognition receptors. However, if they are not fully ingested by macrophages due to foreign body production exceeding the removal capacity of macrophage or stress-induced immune suppression, their removal fails, which results in development of various diseases.

Appropriately regulated macrophage activation first enhances the capacity of foreign body elimination. It has another advantage: acquisition of stress resistance. The safest way to activate macrophages is oral ingestion of a food with a long meal experience. We screened various foods, and found wheat flour has an ability to activate macrophages and the active ingredient is lipopolysaccharide (LPS) derived from wheat-symbiotic gram-negative bacteria *Pantoea agglomerans*. Oral or nasal administration of *Pantoea* LPS is useful in humans and mice for preventing the onset of hyperlipidemia, diabetes, and atopic dermatitis as well as their treatment. In addition, it shows preventive and treatment effect on viral diseases including mouse Aujeszky's disease, koi herpesvirus disease in carp, and human herpes.

In this seminar, as part of clarifying usefulness of oral or dermal administration of *Pantoea* LPS through their various effects in dogs, we present our first analysis of its effects in 153 cases of canine atopic dermatitis. Then, we show another analysis of its effect in 101 cases of canine parvovirus (CPV) disease, which does not have a conventional treatment method and is associated with a high fatality rate.

### ATOPIC DERMATITIS

Dogs diagnosed as having atopic dermatitis or allergic dermatitis (based on medical history, skin symptoms, and diagnostic criteria by the International Task Force on Canine Atopic Dermatitis) were enrolled. Supplement containing *Pantoea* LPS "LPS Dr" (Scarecrow) was used. One hundred and twenty-seven cases had concomitant medications including steroids and antihistamine and antibacterial agents. Twenty-six cases were free from concurrent drugs. Ten to 20 µg/kg/day of *Pantoea* agglomerans was administered for 1–2 months with a meal or alone. If the dog was on medication, the drug was administered concurrently but other supplements were not administered. Then their symptoms were evaluated before and after administration.

In 153 cases receiving oral administration of this LPS supplement, significant effect was seen in 23 cases and effectiveness in 67 cases. Improvement effect was seen in a total of 90 cases (58.8%). Severe adverse reactions were seen neither in 58 cases (37.9%) with no obvious improvement nor in five cases with deterioration (3.3%). Effectiveness of LPS was seen statistically significantly more in male dogs (improvement rate: 69.1%) than in female dogs (improvement rate: 50.6%) ( $P = 0.002$ ) (Fig 1). The improvement rate was 80% in Chihuahua, while it was 40% or less in Shih Tzu and toy poodles. This result suggests that effectiveness of LPS depends on the type of dog. From these findings, LPS derived from *Pantoea agglomerans* is thought to be a potentially effective supplement for atopic dermatitis in the treatment of canine allergic disorders.

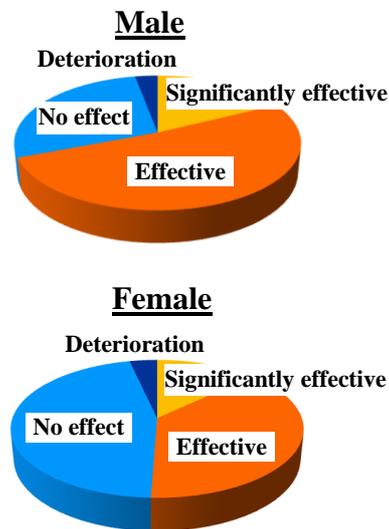


Fig 1. Comparison of the effect of 2-month LPS administration between male and female dogs with atopic dermatitis

### CANINE PARVOVIRUS DISEASE

Canine parvovirus disease (CPVD) spread throughout the world including Japan in the latter half of 1970s. Due to its high fatality rate and contagiousness, it is still a serious problem in breeding places and pet shops. In Southeast Asian countries, many young dogs die from CPVD. Immediate establishment of surefire cure and an effective prevention method have been strongly desired.

We extracted 101 identifiable cases from the dogs judged to be having CPVD and asked for them to be admitted to D&C Veterinary Clinic from No-

vember 2010 to April 2013, and included them in the study. Diagnosis was confirmed by clinical symptoms and CPV antigen (ELISA or PCR). Treatment was performed according to the textbook. To be more precise, fluid administration, Stronger Minophagen C, vitamin Bs, Neurotropin, antibiotics (ampicillin, Tribriksen, Baytril, etc.), INTERCAT, antiemetics, Tamiflu, and constipating agents were used. Pantoea LPS “LPS Dr” was used appropriately.

In consequence, overall treatment effects in terms of survival rate were similar between INTERCAT, Tamiflu, and LPS. The survival rate was 50% or less with any of them. While the survival rate after treatment was 85.7% (six out of seven cases) in the group with LPS administration before admission, it was 55.56% (five out of nine cases) in the group without LPS experience before admission (Fig 2).

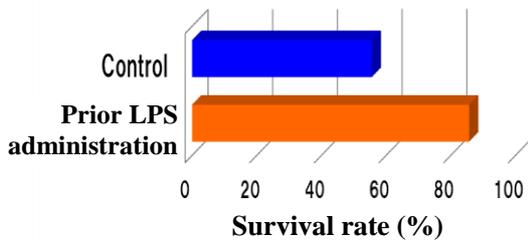


Fig 2. Survival rates of LPS group (with LPS administration before CPVD determination) and non-LPS group (control).

### CONCLUSION

Pantoea bacteria do nitrogen fixation and ionize inorganic phosphorus. They form a symbiotic relationship with many plants including wheat, rice, sweet potato, apple, and pear around the world. Fermented rye bread, consumed as a health food widely in Europe and America, is manufactured by lactic acid fermentation after proliferation of Pantoea agglomerans. Biologics using living Pantoea bacteria are also developed to protect apple and pear fruits from fungal diseases in Europe. From these facts, it is thought that Pantoea agglomerans are safe gram-negative bacteria with a long meal experience.

LPS exists in a substance located on the cell membrane of gram-negative bacteria. It can activate macrophages in vitro at 5 pg/ml or more. Peptidoglycan, a major immune activator of Lactobacillus, exerts macrophage-activating effect at 1µg/ml or more. It shows that LPS activates macrophages extremely

(10,000 times or more) efficiently. Recently, many findings have been collected showing that humans maintain homeostasis and immune balance by taking LPS from the environment. However, LPS intake is decreasing substantially in the current sanitary environment. It would be safe to say that LPS has been consumed as a kind of immune vitamin. It is thought to be relevant that the improvement effect of LPS on atopic dermatitis is attributed to the supplementation with immunizing vitamins, which has been insufficient.

The aim of CPDV treatment with LPS is improvement of natural immunity, or natural immune function for eliminating foreign bodies. This role is mainly played by macrophages. It is also known that LPS induces not only appropriate activation of macrophage but also resistance against various stresses. This antistress effect is thought to be involved in the weight gain of weaning piglets and chicks. Since young dogs have low tolerance for stress and high susceptibility to infections, the antistress effect of LPS is likely to prevent CPV infection in the early stage. From these findings, it is inferred that Pantoea agglomerans LPS enhances preventive/treatment effect against viral diseases via natural immunity.

This study shows for the first time that early LPS administration has effectiveness against canine parvovirus disease. Further gathering of data about safety and other various biological reactions will be required, but LPS administration is worth applying to not only CPV infection but also other communicable diseases.