

Usefulness of Oral Administration of Lipopolysaccharide for Disease Prevention Through the Induction of Priming in Macrophages

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Abstract. Many publications show that macrophages are closely involved in etiology of diseases, such as cancer, diabetes, arteriosclerosis, and Alzheimer's disease. Recent studies show that waste products (e.g. dead cells, denatured proteins, oxidized lipids, and advanced glycation end-products) are the real causative agents of lifestyle-associated diseases. From the standpoint of health maintenance, macrophages eliminate foreign objects and waste products from an animal's body and appear to be quite important for maintaining homeostasis. There are two stages of activation of macrophages: one is priming and the other is the triggering stage with cytokine secretion. The priming stage of macrophages is an ostensibly functional stage without characteristic morphological changes and secretion of cytokines, but it functionally promotes clearance of waste products. In this review, we discuss the usefulness of oral administration of lipopolysaccharide (LPS) as a macrophage-priming agent for prevention/treatment of several diseases, including cancer. Moreover, the oral administration of LPS is safe. These observations suggest that LPS may be considered a vitamin-like substance with therapeutic properties.

Based on pathological analysis, macrophages are believed to be causative cells of many lifestyle-associated diseases, as well as inflammatory and allergic diseases, including cancer.

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Macrophages mainly recognize and eliminate waste products, such as apoptotic cell bodies (1), denatured proteins (2), oxidized lipids (3), advanced glycation end-products (4), cancer cells, and atypical cells by means of various recognition receptors. On the other hand, when these waste products remain for a long time in the body, they cause chronic inflammation in tissues, leading to several diseases. This elimination function of macrophages is the starting point of maintaining a healthy state of a living body.

In general, activation of macrophages is accompanied by production of cytokines or reactive oxygen species, but the priming stage of silent activation does not involve production of cytokines or reactive oxygen species, nor morphological changes (5). This stage enhances the elimination of encountered foreign objects and waste products. In this review we focus on the role of priming macrophages in maintaining host health conditions using oral administration of LPS.

Significance of Priming Macrophages in Health Maintenance

There is some speculation about the usefulness of the priming stage because it promotes efficient elimination of invasive viruses or bacteria, and prevents the development of infectious diseases. For example, tubercle bacilli can survive in the phagosome after phagocytosis by macrophages. In general, the pH of the vacuole declines to 5 after maturation, but if tuberculosis bacilli suppress lowering of the pH and fusion of a lysosome with a phagosome, then an active infection may start (6). On the other hands, a primed macrophage induced by interferon- γ (IFN- γ) could easily digest the tubercle bacilli in phagolysosomes, thus preventing such infection.

It is reported that the priming stage escapes immune suppression caused by severe stress. As a marker of innate

immune activity in mice, we analyzed serum tumor necrosis factor- α (TNF- α) activity 90 min after intravenous administration of OK-432 (Picibanil; Chugai Pharmaceutical) 0.1 KE (1KE=0.1 mg dry *Streptococcus pyogenes*) (7). Surgical stress induced by 30 min of laparotomy caused severe suppression of serum TNF- α activity (to less than 1% of normal level). When the priming stage was induced 3 h before surgery, surgery-induced TNF- α suppression was avoided, but this approach was not effective immediately before the surgery. Although no dead mice were observed after intraperitoneal administration of *Staphylococcus aureus*, 67% of the mice died after laparotomy. The induction of priming 3 h before a surgical procedure significantly reduced the percentage of dead mice (11% death rate) (7). This phenomenon showed that the priming stage helps to prevent suppression of innate immunity caused by severe stress.

Significance of Oral Administration of LPS in Health Maintenance

In terms of disease prevention, food appears to be more useful than medicine for maintenance of macrophage functions. Until now, several functional foods such as mushrooms, yeast, and lactic acid bacteria have been known to activate macrophages. Moreover, we paid attention to LPS of Gram-negative bacteria as a functional material in many foods (8). Although LPS has been considered a toxin in the medical field, oral and dermal routes of LPS administration did not lead to the adverse effects of an endotoxin (9). In addition, LPS are abundant and structurally diverse in the environment, although long-studied LPSs are at the frontier of biological research (10).

LPS has rarely been used clinically because intravenously administered LPS behaves as an endotoxin that causes a cytokine storm, leading to systemic inflammation, sepsis-related toxicity, and septic shock. On the other hand, we have found that orally or dermally administered *Pantoea agglomerans* LPS (LPSP) has positive effects such as being antidiabetic (11, 12), anti-allergic (13), anti-dyslipidemia (14), and anti-tumor effects (15). Nonetheless, the safety of oral or dermal administration of LPSP is unexpectedly high (9). Thus, these administration routes may be suitable for disease prevention (13, 16).

The effects of oral administration of LPS are markedly different from those after intravenous administration, which causes a cytokine storm with systemic inflammation, such as endotoxic shock. The mechanism of action of orally-administered LPSP remains enigmatic. However, the effects appear to be mediated by a mechanism different from that of the intravenous route because LPSP cannot be detected in the serum and does not induce cytokine production after oral administration of LPSP in mice (data not shown). The

intravenous and oral routes of administration are analogous to serum-containing and serum-free conditions, respectively, suggesting that our study can be used as a starting point for analyzing the mechanism of action of orally administered LPSP.

Induction Priming by Oral Administration of LPS

The central role of phagocytes is the elimination of foreign objects and waste products from the body of an animal. In mammals, macrophages are known as professional phagocytes. Substances that have an ability to induce priming in macrophages are known as macrophage-activating factors such as IFN- γ ; TNF- α , an immune complex; and LPS. Because there is no apparent change in morphology and secretion of cytokines and reactive oxygen species, macrophages at the priming stage, under the influence of secondary stimuli, are believed to produce cytokines or reactive oxygen species or to initiate phagocytosis.

The transcriptome of the priming stage of macrophages was analyzed and research regarding IFN- γ -mediated signal transduction showed up-regulation of signal transducer and activator of transcription 1 (Stat1) and down-regulation of Stat3. Furthermore, according to the report by Deng *et al.*, as little as 5 pg/ml of LPS could prime bone marrow-derived macrophages (5). In this model, an extremely low dose of LPS caused disintegration of reticuloendotheliosis viral oncogene homolog B (RelB), through Interleukin 1 receptor associated kinase 1 (IRAK1) and Toll interacting protein (Tollip) activation. Although the molecular mechanisms underlying the priming stage have yet to be characterized, one possible regulatory mechanism for this phenomenon is disinhibition of the nuclear factor-kappa B signaling pathway.

LPS Use in the History of Medicine

Coley's toxin has been used as an immunotherapy in patients with cancer since 1891, and this practice is continued even today because of positive experience gained from more than 120 years of use. Coley's toxin contains a mixture of dead *Serratia marcescens* and *Streptococcus pyogenes* bacteria and is known as a LPS-containing therapeutic agent (17).

Next, *Escherichia coli* (strain Nissel 1917), which was isolated as a Gram-negative bacterium from feces from a soldier approximately 100 years ago, has a long history of use in probiotics against colitis, diarrhea, inflammatory bowel diseases, and diverticulosis worldwide (18). This bacterium also contains LPS. A prescription is required for this probiotic in Europe and North America.

Furthermore, Eksalb and Posterisan are topical ointments developed in Germany (Dr. Kade Pharma) for skin diseases/injuries (such as eczema and burns) and hemorrhoids.

Table I. Gram-negative bacteria lipopolysaccharide (LPS) used for medical purposes.

Medicine name	Applications	Bacterial component	Year introduced (Other country)
Corey's toxin Mutaflor (<i>E. coli</i> , Nissel 1917)	Cancer Colitis, diarrhea, inflammatory bowel diseases, diverticular (Probiotics)	<i>Serratia marcescens</i> , <i>Streptococcus pyogenes</i> <i>Escherichia coli</i>	1891, in USA 1917, in Germany (US, Canada, etc.)
Eksalb	Skin disease	<i>Escherichia coli</i> , <i>Staphylococcus</i> , <i>Pseudomonas aeruginosa</i> , <i>Streptococcus</i>	1962, in Japan (Germany)
Posterisan	Hemorrhoid	<i>Escherichia coli</i>	1965, in Japan (Germany)
Cervarix	Adjuvant (HPV vaccine)	<i>Salmonella minnesota</i> lipid A	2009, Japan (USA)

HPV: Human papillomavirus.

Both medicines contain dead *E. coli* cells and a significant amount of LPS (19). Esalb and Posterisan are approved in Japan as medicines and have been sold in pharmacies since 1962 and 1965, respectively.

Recently, GlaxoSmithKline started manufacturing monophosphoryl lipid A (MPL), which is produced from decomposed *Salmonella* LPS lipid A, and which has a weakened biological activity because it is intended to be used in injections. MPL serves as an adjuvant in Cervarix, and the dose of MPL is 50 µg/person) (20). It is believed to have approximately 1/2000 less activity than that of LPS because of the modifications. The above examples of the use of Gram-negative bacteria or LPS as a medicine indicate their utility in various diseases. These data are summarized in Table I.

The Biological Role of LPS

LPS of Gram-negative bacteria was found to be an active component of an endotoxin, and was recognized as a cause of septic shock. Mammals have a strong immune reaction against LPS injected into the body. Based on these observations, LPS was prohibited from use in injectable medical supplies. Nonetheless, recent clinical data indicated that sepsis therapy involving an antagonist of the toll like receptor-4-myeloid differentiation protein-2-complex (receptor of LPS) showed no significant differences between the control and the test group (21).

The mechanism of high sensitivity to LPS has been elucidated and it appears to involve a complicated complex of signaling proteins. LPS first binds to the LPS-binding protein in the blood, and the complex is then transported to the CD14 receptor molecule on the cell surface of macrophages. Finally, LPS is transferred to the receptor complex containing TLR4 and MD2, triggering intracellular signaling events (22).

Considering the purposiveness of the LPS, we believe that LPS may be regarded as a micronutrient with some medicinal properties for the human body rather than as an endotoxin. For example, epidemiological studies show that LPS is inversely correlated with risk of allergic disease (hygiene hypothesis) (23). The knockout of the gene of the LPS receptor in mice results in low resistance to infection (24). Moreover, the amount of LPS in various foods indicates that some herbal Chinese medicines contain a few tens of micrograms of LPS per gram of dry weight (8), whereas health foods contain several micrograms. These data suggest that ingestion of a certain amount of LPS may be important for health maintenance.

Disease-preventive Effects of Oral Administration of LPS

We have studied the preventative and curative effects on various diseases of oral administration of *Pantoea agglomerans* LPS, originally isolated from wheat flour. We observed several effects, such as protection from infectious diseases [bacterial and parasitic (toxoplasmosis)], improvement of diabetes and lipid metabolism (14), antiallergic properties in atopic dermatitis (13), an infection prophylaxis effect (25), and beneficial effects in cancer (26). LPS performs an important function in regulation of intestinal bacterial flora through the induction of bactericidal peptides (27). After oral administration, LPS is delivered to Paneth cells in intestinal crypts and induces production of bactericidal peptides defensins, such as regenerating islet-derived protein 3 gamma (28) and cryptdin-4 (29). According to the report of Masuda *et al.*, cryptdin-4 has the ability to control the growth of pathogenic bacteria in the intestine (29).

Vancomycin-resistant *Enterococcus* (VRE) is a drug-resistant bacterial pathogen (28). VRE is the result of long-term medication with antibiotics, and it is becoming a social problem as an infectious disease of hospitals.

Usually, LPS comes from many foods or Gram-negative bacteria in the intestine. On the other hand, taking many antibiotics prevents production of intestinal bactericidal peptides because of the suppression of the growth of intestinal bacteria. If LPS is not supplied under such conditions, production of bactericidal peptides may not be sufficient for protection from VRE (28). Similarly, abundant oral administration of antibiotics promotes bacterial invasion into the body from the intestinal tract (bacterial translocation) because of the decreased immune potential of the host. It is also reported that bacterial translocation (from the intestine to other tissues) can be maintained at a normal low level by means of orally administered LPS.

We found that oral administration of *P. agglomerans* LPS enhances the effects of an anticancer agent (26). B16 melanoma was transplanted into the abdominal cavity of C57BL/6 mice, and all mice were dead within three weeks. Although a survival advantage was observed after intraperitoneal administration of doxorubicin, all mice were dead within eight weeks. A significant improvement of the survival period was produced by oral administration of *P. agglomerans* LPS given simultaneously with doxorubicin. One of the possible reasons for the protective effect of LPS is that it reverses immune suppression through the induction of antiapoptotic gene, *B-cell lymphoma 2*, to prevent apoptosis of macrophages caused by doxorubicin (30).

In a model of chronic stress based on daily dexamethasone injection in chicken, antibody production against a *Salmonella* vaccine was significantly decreased. Oral administration of *P. agglomerans* LPS improved the antibody titer against *Salmonella* in this chronic stress model (31). It was also shown that oral administration of LPS protects against steroidal withering of immune system organs: the spleen and bursa. These results indicate that oral administration of LPS saves immune cells from death caused by several types of stress.

Conclusion – Macrophages and LPS for Maintaining Health

Macrophages recognize and eliminate oxidized lipids, denatured proteins, advanced glycation end-products, and dead cells. These are the true causative agents of chronic inflammation, which can lead to several diseases such as diabetes, arteriosclerosis, cancer, allergy, and dementia. As mentioned above, the essential role of macrophages in health maintenance is mainly believed to be the removal of waste products from the body. In accordance with this concept, we believe that a new paradigm should be adopted for health maintenance. For example, an outbreak of a disease may be the result of suppression of macrophage function that ensures elimination of waste products.

Although LPS has been described as an endotoxin, Marshall proposed a new function of LPS as an exogenous hormone (32). Meanwhile, we found several similarities of LPS to vitamins. Firstly, a hormone is synthesized within the body, but animals cannot synthesize vitamins nor LPS. Secondly, the history of the discovery of vitamins also has some similarities to that of LPS. Vitamin C and B were discovered as nutritional factors that were noticed because nutrition deficiency in these vitamins resulted in scurvy and beriberi, respectively. In this regard, LPS is known as the most relevant environmental factor whose deficit contributes to the development of allergic diseases according to the hygiene hypothesis. Thirdly, some vitamins and LPS are supplied by intestinal bacteria. According to these data, we consider LPS a vitamin for the immune system rather than an exohormone.

Conflicts of Interest

The Authors have no financial conflicts of interest.

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