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Short Review

Oral Administration of Lipopolysaccharides for the Prevention of Various Diseases: Benefit and Usefulness

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Abstract. *It is well known that intravenous administration of lipopolysaccharide (LPS) induces severe toxicity in mammals. The maximum tolerated dose of intravenous administration of LPS in humans is reported to be only 1 to 4 ng/kg body weight. However, oral administration of a high dose of LPS caused no toxicity or systemic inflammation in other mammals, birds, or fish. Two weeks of oral administration of a high dose of LPS (2 mg/kg) did not induce toxicity in a rat experiment. Moreover, several experiments have reported that oral administration of LPS had preventative and curative properties against various diseases, including allergic, and lifestyle-related diseases. These results demonstrate that mucosal administration of LPS acts via a different regulatory mechanism in biological responses from that of parenteral administration. Mucosal administration of LPS is thought to be quite promising for prevention of diseases, but LPS is rarely used. In order to expand the usage of oral administration of LPS for preventing lifestyle and allergic diseases, it will be necessary to clarify the mechanisms that arouse immune responses after oral administration of LPS. This short review presents a recent observation of the usefulness of orally administered LPS.*

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Structure of LPS

Lipopolysaccharide (LPS) is the major component of the outer membrane of gram-negative bacteria and has amphiphilic characteristics due to its hydrophilic polysaccharide and hydrophobic lipid moieties. Its fundamental structure comprises three parts: (i) lipid A, (ii) core sugar, (iii) and O antigen (O-polysaccharide). Lipid A is composed of 4 to 7 fatty acid chains bound to two glucosamines, and a core sugar part that is composed of 8 carbon sugar, keto-deoxyoctonate (KDO), which is highly conserved among bacterial species. The core region is an oligosaccharide containing characteristic sugar residues, KDO and heptose, and its chemical variation is more limited than that of O-antigen. Lipid A acts as a membrane anchor (Figure 1).

Immunological response to LPS is triggered because of its binding to the receptors for immune cells and some epithelial cells, which causes activation of nuclear transcription factors by intracellular signals. It is generally recognized that CD14 serves as a high-affinity receptor for LPS after catalytic transfer of LPS monomers by LPS-binding protein (LBP) and that of the CD14–LPS complex (1). The role and structure of the toll-like receptors (TLRs) play an important role in innate immunity. Immune cells recognize specific structures present on the pathogen, such as peptidoglycan, lipopolysaccharide, β-1,3 glucan, double-stranded RNA, and non-methylated CpG DNA (1, 2).

The complex of CD14, TLR-4, and myeloid differentiation factor-2 (MD2) has a higher sensitivity that can induce intracellular signals by 0.1 ng/ml concentration of LPS–LBP complex (3). Consequently, proinflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and

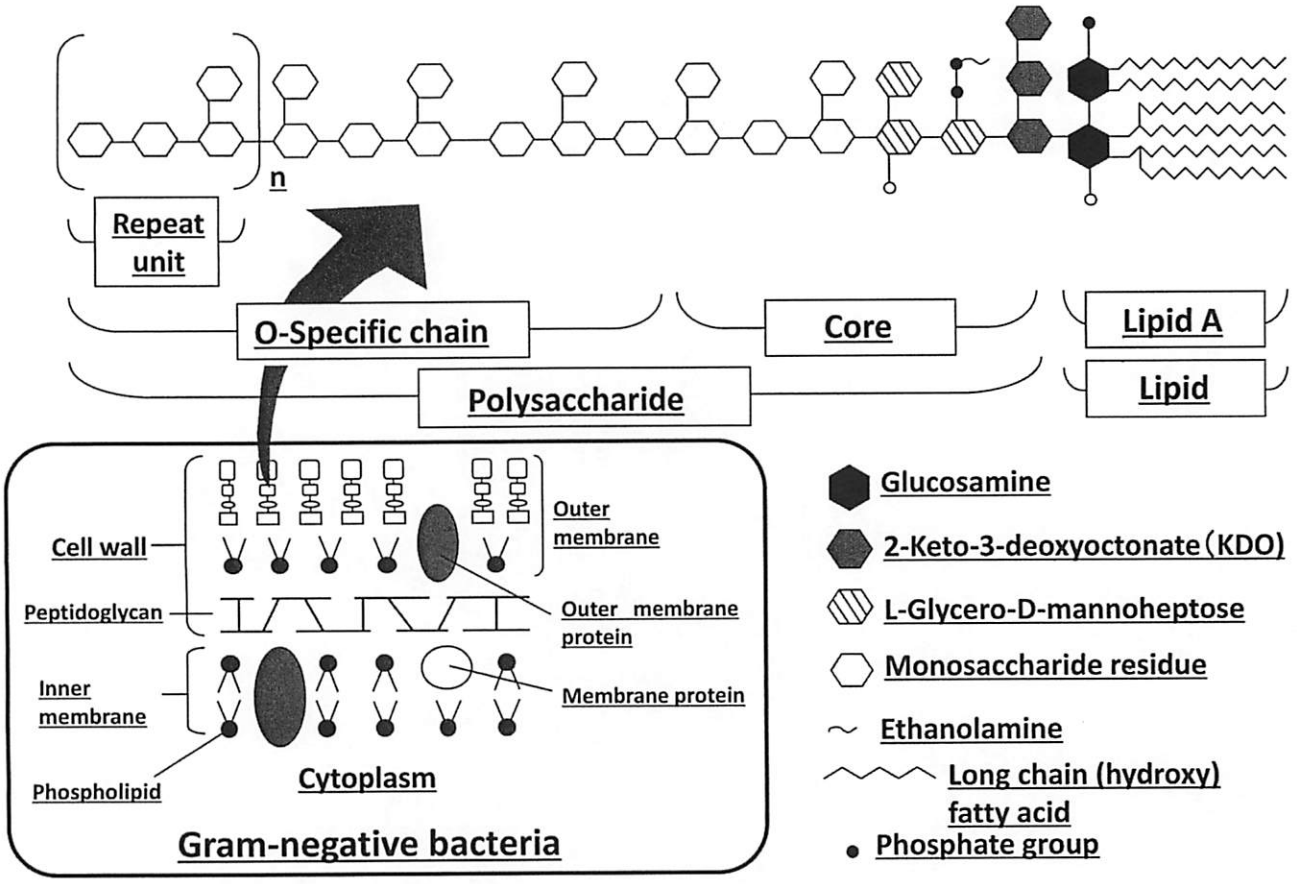

Figure 1. *Fundamental structure of lipopolysaccharide (LPS).*

IL-6 are induced and activated as immune responses by dendritic cells (DCs), T- and B-cells, granulocytes, natural killer cells, and macrophages.

It is believed that the lipid A component of LPS is responsible for these biological activities. However, our recent study of the biological function of LPS using specific monoclonal antibodies against O-polysaccharide components of LPS indicated the importance of the O-polysaccharide chain to LPS function. This function can be assumed to present a lectin-like adaptor molecule associated with receptors for LPS. It may resemble dectins (4), which are known to be the binding molecules for β-1,3 glucan and associating TLR-2. However, to date there is no study that identifies the specific receptors for O-polysaccharide of LPS.

Biological Activity of Intravenous Administration of LPS

Otto *et al.* reported a clinical trial of intravenous administration of LPS exerting an antitumor effect that was investigated in 27 patients with advanced colorectal cancer (5). One complete regression and two partial responses were observed in these patients, however, intravenous injection of LPS induced transient renal and hepatic toxicities. A phase I study defined the maximum tolerated dose of intravenous administration of *Salmonella abortus equi* LPS in humans as being 1 to 4 ng/kg body weight (6, 7). Severe constitutional side-effects, such as fever (World Health Organization grade III), chills, and hypotension, were the dose-limiting toxicities (6, 8). Acute toxicity of intravenous administration of LPS in mice was 4 to 8 mg/kg, with a lethal dose of 50 (LD50) (9). These results demonstrated that intravenous administration of LPS resulted in severe toxicities by causing systemic inflammation, however, some beneficial antitumor effects were anticipated by activation of innate immunity.

The highly sensitive cellular response of immune cells to LPS observed *in vitro* also illustrates an evoked immune response *in vivo* with intravenously administered LPS. When LPS is administered intravenously, it causes a dose-related increase in serum C-reactive protein, TNF-α, IL-1β, and IL-6, which further causes severe fever, diarrhea, vomiting, and hypotension (10). Intravenous administration

of LPS after pretreatment with dichloromethylene-diphosphonate (Cl_2MDP)-liposomes resulted in a significant reduction in mortality, *i.e.* from 55% to 14% (11). Therefore, the pathogenesis of lethal toxicity of LPS is due to systemic overexpression of proinflammatory cytokines from activated macrophages.

As for the fate of LPS, LPS was measured in plasma within a few minutes after intravenous administration, and most LPS was transported to the liver for metabolic degradation. A small amount of plasma LPS was metabolized in the spleen, lungs, kidneys, and adrenal glands, and further excreted in the feces (12).

Biological Effects of Oral Administration of LPS

Oral administration of LPS demonstrates completely different results when compared to parenteral administration. Oketani *et al.* stated that oral administration of LPS is not harmful to animals (13). Schryvers *et al.* found no evidence of LPS toxicity with 20 μg/ml intake after 40 days in mice (14). Illyés *et al.* reported that repeated oral administration of high doses of *Escherichia coli* LPS had no demonstrable effect on small intestinal structure and cell proliferation in rats (15). We found that high doses of single oral administration of *Pantoea agglomerans* LPS (600 mg/kg) had no side-effects in rats (16). Moreover, oral administration of 300 mg/kg of this LPS, which was almost 30,000 times more than the recommended amount of LPS (10 μg/kg) in animals (human, chicken and fish), for 28 days showed no evidence of hepatotoxicity, nephrotoxicity, inflammation, or weight decrease in rats. These findings demonstrate that oral administrations of LPS are quite safe for animals.

Biological responses evoked because of oral administration of LPS have been reported. Murakami *et al.* reported that B-1 cells derived from the lamina propria in gut and peritoneal cavity were activated by oral administration of *Salmonella* LPS (100 μg/mouse) after 7 days in normal C57BL/6 mice (17). B-1 cells are thought to be a kind of phagocyte because of their ability to uptake apoptotic thymocytes and *E. coli* both *in vitro* and *in vivo* (18), and they possess differentiating potential similar to phagocytes (19). Chen *et al.* reported that oral administration of *E. coli* LPS (10 μg/ml of drinking water) protected against bacterial translocation and peritoneal macrophage suppression caused by the administration of antibacterial drugs in severely burned mice (20). Oral administration of LPS has beneficial properties that protect against intestinal bacterial infections. Masuda *et al.* reported that activated Paneth cells secrete cryptdin-4 (21), which has the most potent microbicidal activity among defensins and may be induced by LPS (22). Rakoff-Nahoum *et al.* demonstrated that oral administration of LPS rescued commensal depleted mice from DSS-induced mortality (23). Márquez-Velasco *et al.* reported that prophylactic oral administration of LPS to mice that underwent cecal ligation and puncture, significantly increased their survival rate and reduced the inflammatory responses in target organs (24).

We have reported that a hot water extract of wheat flour (oral administration) contains macrophage-activating substances derived from concomitant gram-negative plant-associated bacteria such as *P. agglomerans*. LPS of this bacterium is termed as IP-PA1, and is a major macrophage-activating substance (25, 26). Research has demonstrated that it is useful for preventing lifestyle-related, allergic, and infectious diseases in both human and animal models. Oral administration of *P. agglomerans* LPS was useful for preventing hyperlipidemia (rabbits) (27), diabetes mellitus (mice and humans) (28), various infectious diseases (mice and shrimps) (25, 29, 30), and ulcerative colitis (mice) (31), and produces analgesic effects (mice, rats, and humans) (32-34).

Possible Pathways of Oral Administration of LPS through the Intestinal Tract

Benoit *et al.* reported that pure LPS did not pass across the intestinal mucosa *in vitro* (35). However, other reports have demonstrated that detectable amounts of LPS increased after oral administration of LPS in animals (36-38). It is estimated that 0.1 to 0.25% of orally administered LPS can be detected in blood by using ^{125}I-labeled LPS. If 1 mg of LPS administration is absorbed to this ratio, 1 to 2 μg of LPS should mathematically exist in blood (36). This amount is enough to cause significant systemic inflammation in mice by intravenous injection. However, 1 mg of oral administration of LPS showed no increase in free cytokines (unpublished data). From these results, we determined that the absorption mechanism of orally administered LPS in intestine is different from that of intravenous administration. Possible pathways of ingestion of LPS by the small intestine mucosal tract recently reported are summarized in Figure 2 (20, 36, 39-44).

These pathways of LPS translocation may allow its penetration into lymphoid tissues, such as Peyer's patch and mesenteric lymph nodes. However, these translocation pathways do not help to clarify the mechanisms of biological function by oral administration of LPS. To fully investigate the mechanism and fate of orally administered LPS, it will be important to assay the systems to describe the condition of innate immune cells after its administration.

Perspectives on Oral Administration of LPS

LPS is an abundant substrate, for example, almost all foods contain 1 ng to 1 μg of LPS per gram of their weight. Moreover, humans constantly come into contact with huge amounts of bacteria in oral and intestinal mucosa. The estimated number of human commensal bacteria range from

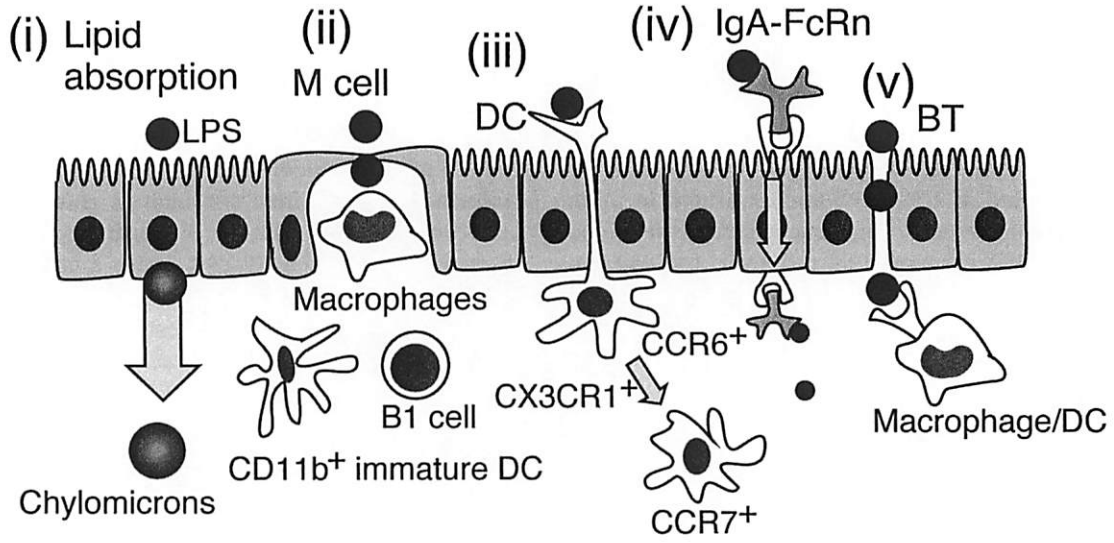

Figure 2. *Possible pathways of ingestion of LPS by the mucosal tract. (i) Lipid absorption and chylomicron formation in intestinal epithelial cell (IEC). (ii) Macromolecule tracking via M cells. (iii) Antigen sampling by dendritic cell (DC). (iv) Transportation of immune complex with IgA by FcRn on IEC. (v) Bacterial translocation (BT) under stressed condition.*

10^3 to 10^{12} per gram of tissue (45). Thus, humans are constitutively exposed to LPS throughout their lives. Some reports indicate that exposure to LPS in this manner may be important for the maintenance of host immune balance (anti-allergic predisposition) (46, 47), and protection from bacterial infections in the intestine (21).

The toxicity of oral administration of LPS is quite low, and many papers provide convincing evidence that support there being various beneficial effects for allergic and lifestyle-related diseases. Thus, in the near future, oral administration of LPS is expected to be used for maintaining animal health. To promote oral usage of LPS, the mechanistic explanation of prevention and cure of various diseases will be needed, but the mechanism to regulate the host's health by oral administration of LPS is not yet clear at all. It is important to discover these underlying mechanisms because it is likely that they are quite different from those occurring with intravenous administration of LPS.

An evaluation method useful for accurate determination of the response to orally administered LPS has not yet been developed. We believe that one possible mechanism of the effect of oral LPS is ascribable to the induction of a priming stage (48). Moreover, recognition of foreign substances (bacteria, viruses, and apoptotic cells) by innate immune cells was up-regulated in the priming stage. In a mouse model, intravenous administration of LPS (0.1-1 ng/mouse) induces the priming stage. This amount of LPS is almost 200,000 times less than the LD_{50} of LPS (200 μg/mouse) (9) and is safe and non-toxic because it does not induce the release of proinflammatory cytokines in mouse blood.

Molecular analysis of a priming stage was indicated by the existence of pro-TNF-α on macrophage membrane (49). Interestingly, pro-TNF-α acts as a ligand and receptor for neighboring macrophage cells, namely the primed macrophages, and they can respond bidirectionally with a reverse signal system (50, 51). Taken together with these data, we propose that the mechanism for maintaining homeostasis by oral administration of LPS includes a signal transfer system via cell to cell contact (termed the macrophage network system) (26, 52).

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