Short Review

The Involvement of O-Antigen Polysaccharide in Lipopolysaccharide in Macrophage Activation

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Abstract. *Bacterial lipopolysaccharide (LPS), which is generally considered to be an endotoxin, is the major constituent of the outer membrane of Gram-negative bacteria. The structure of LPS consists of three regions; lipid A, core oligosaccharide and O-antigen polysaccharide (O-PS). The structures of lipid A and core oligosaccharide are highly conserved among bacterial genera, but that of O-PS varies and differs in common bacterial species. Although studies of the biological activities of LPS have mainly focused on the lipid A moiety, a recent study gradually clarified the importance of O-PS to elicit the biological activities. In this review, we summarize previous studies on the correlation between the structure of O-PS and the biological activity of LPS, and discuss the possibility of innovative drug development using modified and synthetic LPS.*

Overview of the Correlation Between the Structure and Function of Lipopolysaccharide(LPS)

LPS exists virtually throughout the environment and can be found in air, food, water, skin and intestine (1, 2). However, LPSs can cause severe damage to the host immune system through systemic inflammation (known as endotoxin shock), which occurs under specific conditions, such as when LPS is administered intravenously. On the other hand, LPSs can also regulate enteric immunity by oral administration, without serious side effects (3). Therefore, LPS has now been re-recognized as exohormone capable of regulating homeostatic balance in relation to the external environment (1).

LPS consists of three regions: lipid A, the core oligosaccharide and O-antigen polysaccharide (O-PS). The lipid A moiety is highly conserved among bacteria and is known to elicit biological activities in immune cells, especially macrophages (4, 5). These findings were verified using various synthetic types of lipid A which elicit different biological activities, such as induction of inflammatory cytokine production (6-10). Moreover, a few reports have focused on the correlation between polysaccharides and biological activities (11). The functional roles of polysaccharides have usually been investigated using rough type (short sugar chain) or smooth type (long sugar chain) LPSs derived from *Salmonella* (12). Jiang *et al*. reported that the smooth and rough type LPSs induce expression of different levels of tumour necrosis factor-α (TNF-α) and interferon-β (IFN-β) because of differences in LPS-binding proteins (LBPs), cluster of differentiation-14 (CD14) and toll-like receptor 4 (TLR4)-dependent signalling, respectively (13). In addition, Huber *et al*. reported that rough type LPS was immediately transferred to TLR4-myeloid differentiation-2 complexes on the cell surface, whereas the smooth type LPS requires further molecules, such as LBPs and CD14, for such transfers to occur (14). These reports suggest that cytokine production is modified by O-PS in LPS, and this phenomenon appears to be caused by the chain length of O-PS. In summary, it can be said that O-PS in LPS is able to elicit biological activities based on reports that state the interactions between O-PS and transporter proteins.

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O-PS Potentially Acts as Direct Modulator for Macrophage Activation

In a pilot study, we investigated the correlation between O-PSs and biological activities using LPSs derived from *Pantoea agglomerans* (LPSp) and from *Escherichia coli* O111:B4 (LPSe, known as conventional LPS), which have almost the same structure as lipid A (15). The study showed that the level of production of interleukin (IL)-12 and IL-17 differed in human peripheral blood mononuclear cells (PBMCs) following incubation with LPSe and LPSp, which led us to hypothesize that O-PS affects biological activities through an unidentified receptor present on the cell surface. It has been reported previously that antibodies against O-PS of LPSp can be used to detect the amount of LPSp (clone: 4E11, 34G2) (16). Different LPSs usually have different molecular weights depending on their diversity, thus making it difficult to analyze their function. Therefore, it is considered that these antibodies are a good tool for directly clarifying the influence of O-PS.

As mentioned above, LPSe displayed broad and ladder bands on sodium dodecylsulfate-polyacrylamide gel electrophoresis analysis due to heterogeneity of its polysaccharide, but LPSp displayed mainly two bands in that analysis. These two bands from LPSp were purified by gel filtrated chromatography and designated as high molecular weight LPSp (HM-LPSp) and low molecular weight LPS (LM-LPSp), respectively (Figure 1). These LPSs appeared to have the same lipid A structure because of their same bacterial origin, suggesting that they are indeed good tools for analyzing the function of polysaccharides compared to previous reports.

Our group then examined the biological activities of HM-LPSp and LM-LPSp using O-PS-specific monoclonal antibodies (IgG and IgM). We found that these monoclonal antibodies suppressed the production of TNF-α and Nitric Oxide in RAW264.7 cells following incubation with HM-LPSp, whereas this was not the case following incubation with LM-LPSp (data not shown). These *in vitro* studies suggest that the biological activities of O-PS can directly modulate the effects of LPS. In addition, we also found that IgG suppressed TNF-α production more than IgM. Therefore, these findings are not the result of steric hindrance of the antibodies.

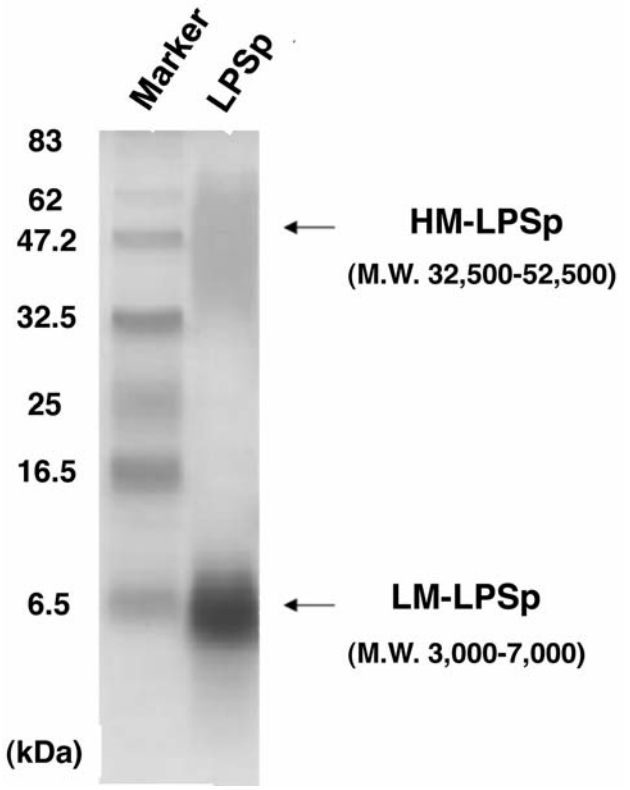

Figure 1. *Silver-stained profile of Lipopolysaccharide derived from Pantoea agglomerans (LPSp). LPSp sample (10 μg) was separated by Tricine sodium dodecylsulfate -polyaclylamidegel electrophoresis and stained with commercial silver staining kit. LPSp was separated mainly into two bands of high molecular mass (from 32.5 to 52.5 kDa) and low molecular mass (from 3 to 7 kDa), named HM-LPSp and LM-LPSp, respectively.*

The Possible Existence of Novel Receptors for O-PS in LPSp

It is well known that lectins have a wide variety of important roles, including cell adhesion, cell signalling, immune response, host pathogen interactions and control of cellular growth. Recently, C-type lectin receptors (CLRs) and C-type lectin receptor-like domains (CLRDs) have been reported to function as adaptor molecules modulating TLR signalling. CLRs are a type II transmembrane protein with a carbohydrate recognition domain to recognize the sugar structure in a calcium ion-dependent manner. Many molecules have been shown to convey signals *via* immune receptor tyrosine-based activation or inhibitory motif (ITAM or ITIM) regions in intracellular domain. It has been reported that ITAM or ITIM signalling could positively or negatively regulate TLR responses in macrophages (17-20). Therefore, it is considered that CLRs or CLRDs are candidate receptors for O-PS, and the biological activities of O-PS possibly mediate CLR or CLRD signaling. Table I shows that the major CLRs and CLRDs are suggested to be involved with TLRs. Although the structure of O-PS in LPSp includes repeating units of rhamnose and glucose (15), the previously reported CLRs or CLRDs have no specificity for these. This suggests that the biological activities of O-PS in LPSp are possibly mediated by unknown or uncharacterized receptor(s), including lectins.

Table I. *A summary of the C-type lectin receptor (CLR)-mediated toll-like receptor (TLR)s expressed in macrophages.*

CLRs (Group)	Name	Localization	Ligand specificity	Regulation factor	Function in macrophages (mainly involvement of TLRs)	Reference
Group 1 (mannose receptor family)	MMR (CD206)	DCs, LC, Mo, Mϕ, LE	Mannose, fucose, GalNAc, sulphated sugars *via* cysteine rich domain.	↑PGE, IL-4, IL-10, IL-13 ↓IFN-γ, LPS	Endocytosis, antigen uptake, cell adhesion	(21-24)
Group 2 (asialoglycoprotein receptor family)	DC-SIGN (CD209)	DCs, HC, dMϕ, aMϕ	Mycobacteria, fungi, viruses	↑IL-13 ↓LPS	Modulates TLR3,4,5-induced cytokine responses	(25-27)
	SIGN-R1 (CD209b)	pMϕ, LSE, LNsE,	Glycans from different pathgens, dextran, *Streptococcus pneumonia* CPS, HIV, ICAM-3	unknown	Associates with the TLR4-MD2 complex to enhance signal transduction in response to LPS	(28, 29)
	M-ASGP-BP (Clec10A)	pMϕ, myeloid DCs	Gal/GalNAc unit	↓LPS	Endocytosis, the mRNA expression of M-ASGP-BP is down-regulated by the LPS-mediated TLR4 pathway involving NF-κB activation	(30, 31)
	Dectin-1 (β-glucan receptor)	Mϕ, DC, PMN, T-cell	Fungi, β-1,3 and β-1,6-linked glucans	↑IL-4, IL-13 ↓IL-10, LPS	Phagocytosis, antigen uptake, cell adhesion, TLR2-independent signal transduction, the role of dectin-1 in vivo is unresolved	(32-34)
	Mincle (Clec4e, Clecsf9)	pMϕ,	Mannose, TDM, Gal/GalNAc Candida albicans, pneumococcal pneumonia, influenza A virus	↑LPS, NF-α, IL-6, IFN-γ	Transcriptional target of NF-IL6 and C/EBPβ, Up-regulated in the lungs of mice infected with pneumococcal pneumonia or influenza A virus	(19, 35-38)

MMR: macrophage mannose receptor; DC-SIGN: dendritic cell-specific intracellular adhesion molecule-3-grabbing non integrin (SIGN); SIGN-R1: SIGN-related 1; M-ASGP-BP: macrophage asialoglycoprotein-binding protein; Mincle: macrophage-inducible C-type lectin, DC: dendritic cell; LC: Langerhans cells; HC: Hofbauer cells; LE: lymphatic endothelium; LNsE: lymph node sinuses endothelium; LSE: liver sinusoidal endothelium; Mϕ: decidual, alveolar, peritoneal macrophages; Mo: monocytes; PMN: polymorphic nuclear cells; TDM: trehalose-6,6-dimycolate (mycobacterial cell wall glycolipid that is the most studied immunostimulatory component of *M. tuberculosis*); GalNAc: N-acetyl galactosamine; HIV: human immunodeficiency virus; CPS: capsular polysaccharide; IL: interleukin; ICAM: intercellular adhesion molecule; PGE: prostaglandin E; IFN-γ:interferon-gamma; NF-κB: nuclear factor-kappa B.

Prospective Study

As mentioned above, the activation of macrophages by LPS was found to be depended on the lipid A structure. However, our data and recent studies support the fact that O-PS in LPS potentially plays a crucial role in eliciting biological activities through a novel receptor. Importantly, under various immune conditions, each tissue macrophage remarkably displays pleiotropic properties (39, 40). Therefore, there is a possibility that the involvement of O-PS in different biological activities is different in each tissue macrophages. It is recognized that LPS derived from the environment exerts beneficial effects through the mucosal immune system (41, 42). On the other hand, it is well known that macrophages and dendritic cells distributed in mucosal tissue have an immune tolerogenic property so as not to cause unnecessary inflammation in host (43-48). In our previous studies, it was reported that the resident intestinal macrophages (IMs) stimulated with LPS did not produce TNF-α, but those pre-treated with immunoglobulin did (49). This suggests that IMs potentially respond to LPS like other tissue macrophages. In addition, we also reported that orally administered LPS prevents various diseases (50-55). Therefore, LPS distributed in the environment undoubtly affects biological activities through the mucosal immune system.

These facts also led us to hypothesize that IMs also have uncharacterized receptors which recognize the O-PS in LPS. The analyses of the interaction between O-PS in LPS and macrophages should establish a foundation for regulating homeostasis. In the future, such studies would lead to creation of novel drugs by the application of modified LPS adapted to individual immune conditions.

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