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Modulation of Mucosal Immune Response by Bacterial Lipopolysaccharide in Nasal Vaccination Models

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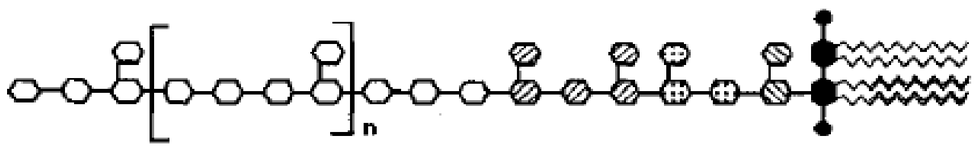
Background

Lipopolysaccharide (LPS) is the major structural component of the outer membrane of gram-negative bacteria and is composed of three distinct domain; lipid A, a core oligosaccharide chain, and an O-antigen. LPS is a potent activator of cells of the immune and inflammatory systems, including macrophages, monocytes, and endothelial cells. LPS activates cells through TLR4 with the accessory proteins CD14 and LPS binding protein.

A schematic structure of bacterial lipopolysaccharide



Objective

To examine the effects of LPS on mucosal and systemic immune responses in intranasally immunized mice.

Materials and Methods

Mice: C57BL/6J, 6-8 weeks old

Mice were divided into six groups (Table 1.), 5 mice per group.

1. Detection of Ag-specific Ab production.

Serum, saliva, and nasal wash fluids (NWs) were subjected to determine the antibodies titer by an enzyme-linked immunosorbent assay (ELISA).

2. Enumeration of Ab-forming cells (AFCs)

Mononuclear cells from the spleen, submandibular glands (SMGs) and nasal passages were subjected to an enzyme-linked immunospot (ELISPOT) assay to detect numbers of AFCs.

Immunization schedule

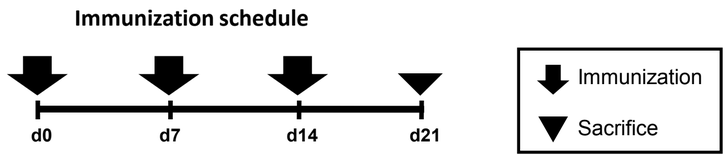


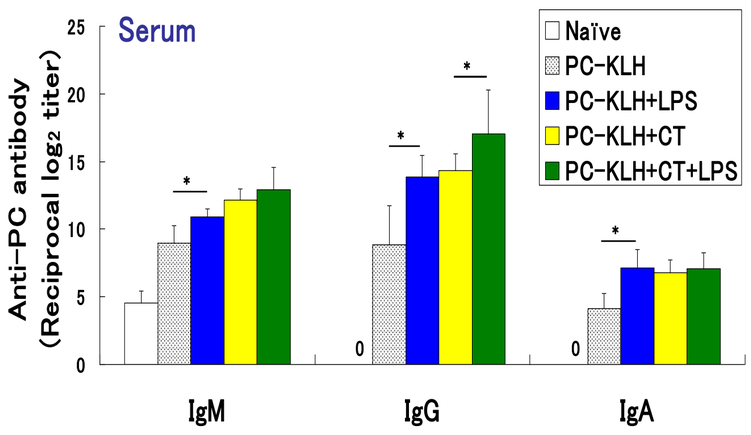
Table 1. Vaccine formulations (per mouse)

Group	PC-KLH (μg)	TNP-KLH (μg)	CT (μg)	LPS (μg)
PC-KLH	50	0	0	0
PC-KLH + LPS	50	0	0	10
PC-KLH + CT	50	0	1	0
PC-KLH + CT + LPS	50	0	1	10
TNP-KLH + CT	0	10	1	0
TNP-KLH + CT + LPS	0	10	1	10

PC: phosphorylcoline, TNP: trinitrophenol, KLH: keyhole limpet hemocyanin, CT: cholera toxin

Results

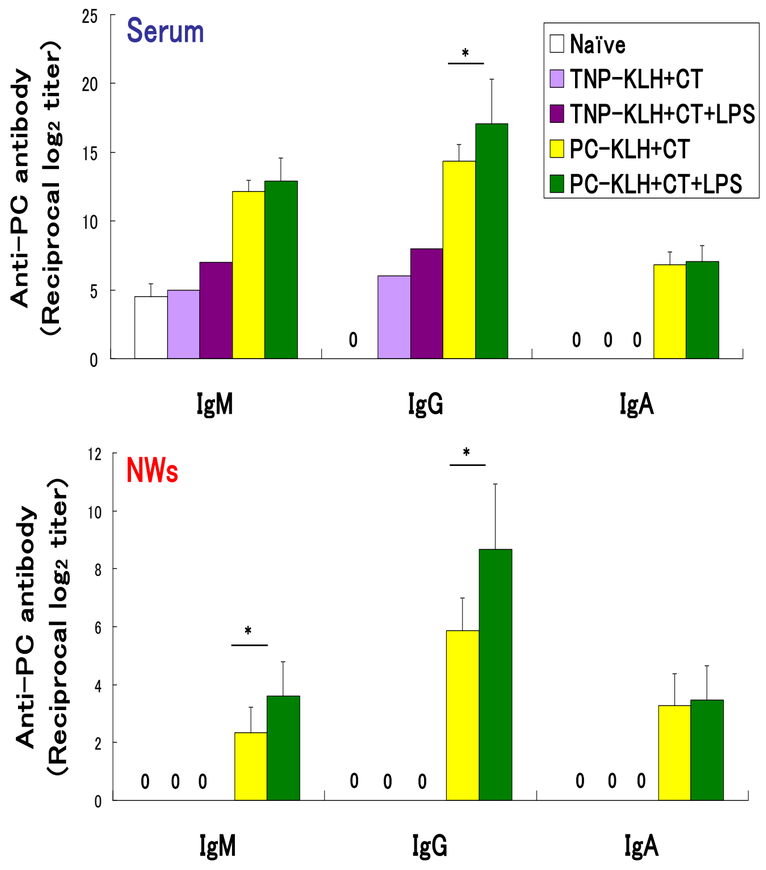
Fig. 1. Anti-PC antibody responses induced by intranasal administration of PC-KLH and CT as an adjuvant in the presence and absent of LPS .



*, $p < 0.01$

The levels of anti-PC antibody responses in serum, saliva, and NWs were determined by ELISA using PC-BSA coated plates.

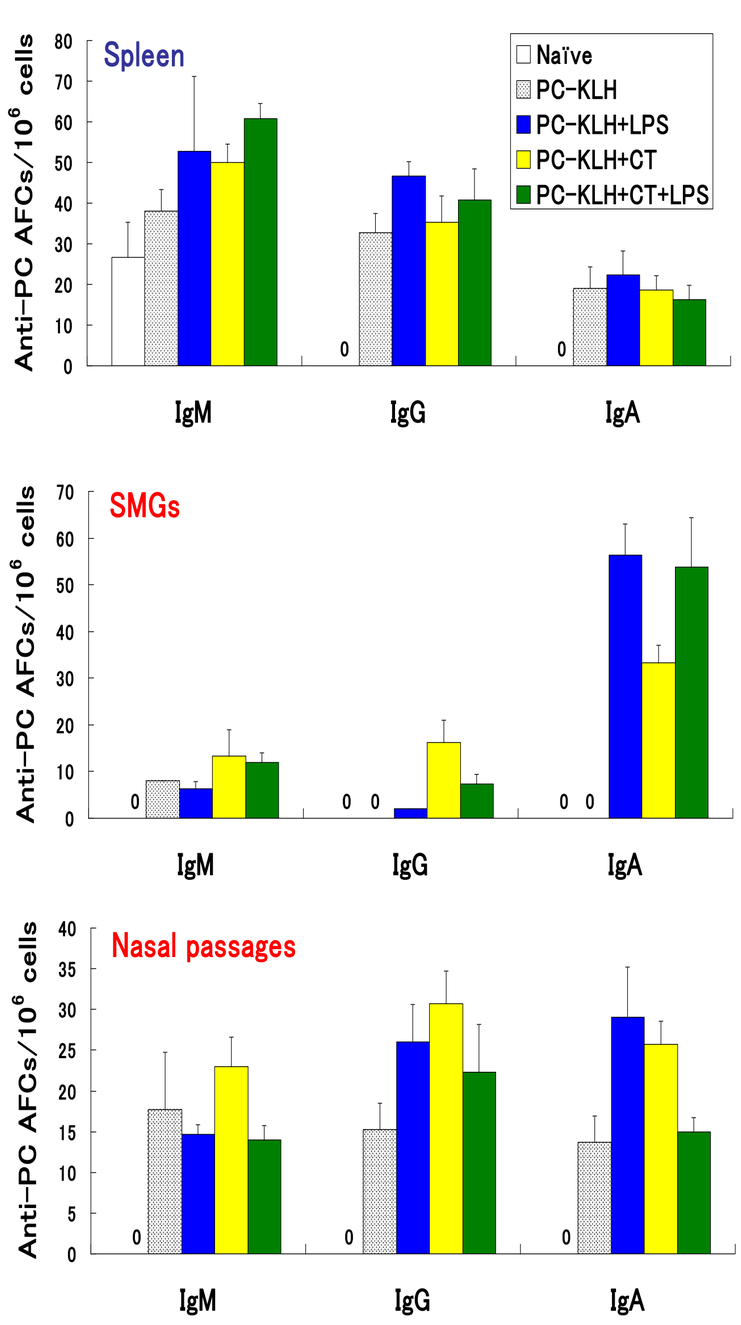
Fig. 2. PC-specific augmentation of antibody production induced by co-administration of LPS with mucosal vaccine.



*, $p < 0.01$

The levels of anti-PC antibody responses in serum, saliva, and NWs from mice immunized with PC-KLH or TNP-KLH were determined by ELISA using PC-BSA coated plates.

Fig. 3. The number of anti-PC Ab producing cells in systemic and mucosal lymphoid tissues



Antibody producing cells were visualized and counted in ELISPOT assay using PC-BSA coated microcellulose membrane.

Discussion

Mucosal and systemic immune responses induced by intranasal immunization with PC-KLH and CT as mucosal adjuvant have been investigated by Tanaka et al (2007). In this study, we examined the effect of co-administration of LPS on systemic and mucosal anti-PC Ab responses (Fig. 1). The adjuvanticity of LPS was found to be similar to CT.

Co-administration of LPS with PC-KLH plus CT promoted anti-PC Ab responses of serum IgG, and mucosal IgM and IgG classes. The administration of LPS with TNP-KLH never increased antigen non-specific anti-PC Ab production, although CT promoted antigen non-specific, IgG anti-PC, to some extent (Fig. 2). Therefore, the increased anti-PC antibody levels by LPS was not likely due to the antigen non-specific polyclonal immunostimulatory property of LPS.

The similarity of LPS to CT in mucosal adjuvanticity was futher supported by ELISPOT assay (Fig. 3), although the increase in IgG anti-PC levels by LPS found in ELISA was not observed in ELISPOT.

Conclusion

1. Intranasal co-administration of LPS enhanced systemic and mucosal antigen-specific Ab responses induced by nasal vaccination.
2. The adjuvanticity of LPS was apparently similar to CT in the induction of mucosal and systemic Ab responses in the nasal vaccination model.
3. Co-adminstration of LPS with CT enhanced antigen-specific IgG Ab response in serum, saliva, and NWs of PC-immunized mice.