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研究題目：Characteristics of mucosal immune responses toward different haptenic determinants including phosphorylcholine（PC）

目的（Objective）：

Phosphorylcholine（PC）is a structural component of a wide variety of pathogens and an immunodominant epitope on the cell wall of *Streptococcus pneumoniae*. Anti-PC antibody response is known to protect mice from invasive bacterial infection. However, the immune responses induced by mucosal vaccination with PC have not yet been characterized. In this study, I examined the characteristics of systemic and mucosal immune responses to different hapten determinants including PC in nasal vaccination models.

材料および方法（Material and methods）：

Animal and immunization

C57BL/6 mice were lightly anaesthetized and immunized nasally with 50 μg of PC conjugated to keyhole limpet hemocyanin（KLH）or trinitriphenyl（TNP）-KLH plus 1 μg of cholera toxin（CT）in the presence or absence of 10 μg lipopolysaccharide（LPS；*Escherichia coli* O128：B12）. Other groups of mice were given intranasally PC-KLH + CT or PC-KLH + LPS. Mice were immunized three times at weekly intervals in all experiments. On days 0 and 28（2 weeks after the final immunization）, blood and saliva samples were collected. Saliva samples were obtained following intraperitoneal injection of pilocarpine（100 μl of 1 mg/ml）diluted in sterile phosphate buffered saline（PBS）. When the mice were sacrificed, nasal wash fluids（NWs）were obtained by installation of 1 ml PBS on three occasions into the posterior opening of the nasopharynx with hypodermic needle.

Detection of antibodies

PC-and TNP-specific antibody（Ab）titers in the samples were determined by an enzyme-linked immunosorbent assay（ELISA）. Polystyrene microtiter plates were coated with 5 μg/ml of PC-bovine serum albumin（BSA）dissolved in PBS. After blocking with 1% BSA in PBS, 2-fold serial dilution of samples were added and incubated for 2 hours at 25°C. Horseradish peroxidase（HRP）-conjugated anti mouse IgM, IgG, and IgA were appropriately diluted and added to individual wells. The coloring reaction was done in 0.1 M citrate phosphate buffer（pH 4.2）containing 1.1 mM 2,2'-azino bis（3-ethylbenz-thiazoline-6-sulfonic acid）and 0.01% H_2O_2 for 50 minutes at 25°C. End point titers were expressed as the reciprocal $\log_2$ of the highest dilution that gave an OD_{405} nm of 0.05 greater than the background.

結果（Results）：

The levels of antigen（Ag）-specific Ab responses in mucosal secretions.

When mucosal antibody production following nasal immunization was investigated, levels of Ag-specific IgA and IgG Ab responses in NWs（Fig. 1）and saliva（Fig. 2）of TNP-immunized mice were higher than those of PC-immunized mice. In contrast, the level of IgM Ab response in NWs of PC-immunized mice was higher than that of TNP-immunized mice（Fig.1）.

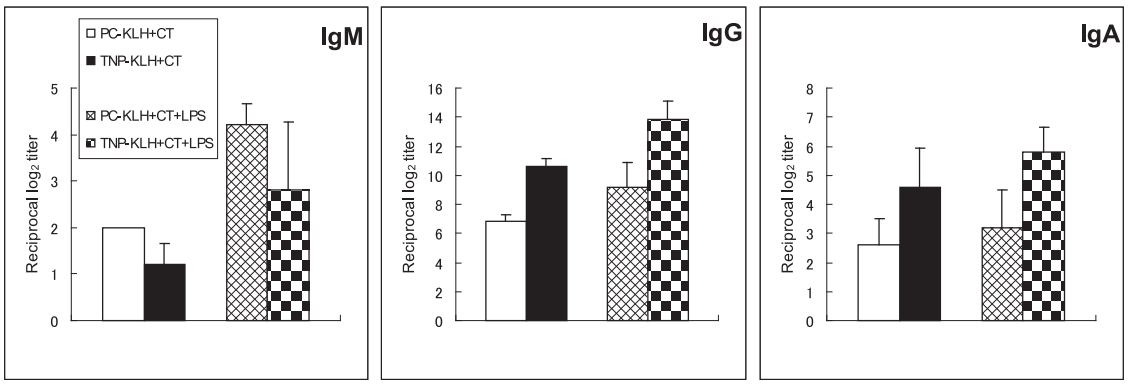


Fig.1 Ag-specific Ab responses in NWs, which obtained 2 weeks after final immunization, of mice immunized intranasally with PC-KLH+CT and TNP-KLH+CT with or without LPS.

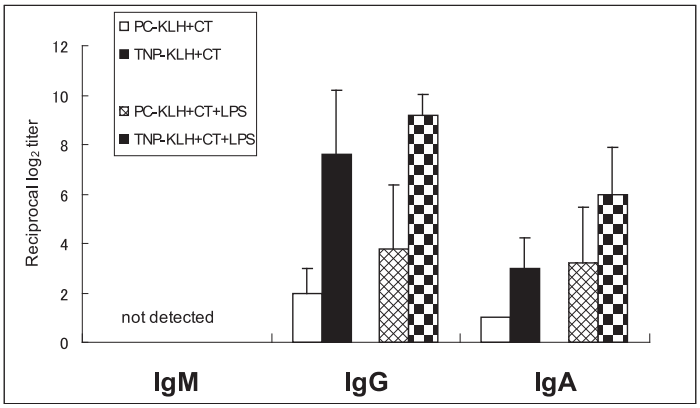


Fig.2 Ag-specific Ab responses in saliva, which obtained 2 weeks after final immunization, of mice immunized intranasally with PC-KLH+CT and TNP-KLH+CT with or without LPS.

The levels of Ag-specific Ab responses in sera.

Systemic Ag-specific Ab responses were found in sera of intranasally immunized mice both with PC and TNP（Fig. 3）. However, the magnitude of increases in Ag-specific Ab levels in PC-immunized mice were tended to be greater than those of TNP-immunized mice, especially for the IgM isotype.

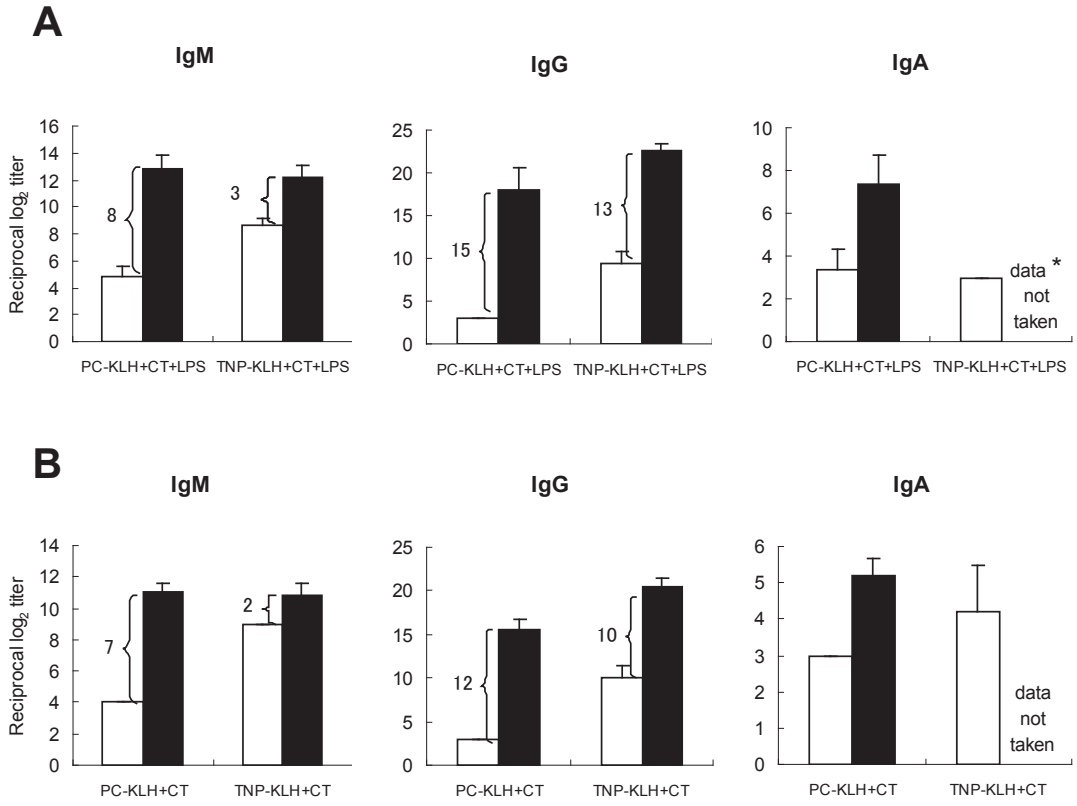


Fig.3 Ag-specific Ab responses in sera, before (d0 □) and after (d28 ■) immunization, of mice immunized intranasally with PC-KLH+CT and TNP-KLH+CT with (A) or without (B) LPS. * TNP-specific IgA Ab responses in sera of TNP-immunized mice were unable to be determined, presumably because very high levels of IgG Ab in samples competed with IgA Ab for binding to the determinant.

考察 (Discussion):

PC is an antigenic determinant expressed on the cell surfaces of certain pathogenic microorganisms including *S. pneumonia* and perhaps also those of some commensals. Therefore, the possibility was considered that immunological memory or tolerance was induced in the animals prior to immunization. In contrast to PC, TNP does not exist in the environment ; an immunological encounter cannot be occurred in animals unless it is artificially administered. The results of the present study showed that the mice possessed the response to PC much the same as to TNP, which suggested the absence of immunological memory or tolerance to PC prior to vaccination. Even higher level of IgM Ab response in NWs and higher magnitude of increases of all isotype Ab in sera were induced by PC-immunization than TNP-immunization, that may intimate a very naïve property of immunity to PC in the untreated mice.

Bacterial LPS is well known to be a polyclonal B cell activator ; it stimulates naïve B cells to differentiate into antibody-producing plasma cells independently from helper T cells, but does not induce an immunological memory state. LPS is a fundamental component of the cell surfaces of gram-negative bacteria and it is thus ubiquitous in the environment. Therefore, it was interesting to determine whether incorporation of LPS into the vaccine formula including

the antigen（hapten-KLH conjugates）and adjuvant CT would alter the immune response induced. However, our results so far indicated only a quantitative augmentation of antibody responses occurred by addition of LPS, which was somewhat unexpected. The influence of LPS in the mucosal immune reaction needs to be investigated more in detail.

成果発表（Research achievement）：

ムルヤトノ　サプタ，片岡宏介，関根伸一，バータルジャフ　ツェルメグ，福井　誠，金川裕子，後藤優樹，伊藤博夫
「経鼻粘膜アジュバント CpG ODN はマクロファージの TLR9 発現と歯周病原性細菌抗原特異的 Th1 免疫応答を誘導する。」
第 60 回日本口腔衛生学会・総会，日本大学松戸歯学部（千葉，松戸），平成 23 年 10 月 9 日

福井　誠，ムルヤトノ　サプタ，後藤優樹，片岡宏介，林田秀明，川崎浩二，前田隆浩，中里未央，齋藤俊行，伊藤博夫
「ヒト唾液中および血中の抗体ホスホリルコリン抗体と動脈硬化リスクとの関連性：五島研究」
第 60 回日本口腔衛生学会・総会，日本大学松戸歯学部（千葉，松戸），平成 23 年 10 月 10 日

Sapta A. Mulyatno, Kosuke Kataoka, Makoto Fukui, Kaname Miki, Rita Orihuela, and Hiro-O Ito
「Modulation of mucosal immune response by bacterial lipopolysaccharide in nasal vaccination models」
第 61 回日本口腔衛生学会・総会，神奈川歯科大学（横須賀，神奈川），平成 24 年 5 月 27 日発表予定