

## 各種病原体由来のワクチン候補抗原および免疫賦活・抑制物質の探索研究

木下 タロウ (大阪大学免疫学フロンティア研究センター)

### Research on vaccine candidates, and immune-stimulating and -suppressive compounds derived from various pathogens

Taroh KINOSHITA, Osaka University

1. Morita YS, Fukuda T, Sena CB, Yamaryo-Botte Y, McConville MJ, Kinoshita T. (2011)

#### **Inositol lipid metabolism in Mycobacteria: Biosynthesis and regulatory mechanisms.**

Biochim Biophys Acta, 1810: 630-641.

Background: Mycobacteria include many medically important pathogens. Their cell wall has many unique features, including the abundance of various inositol lipids, such as phosphatidylinositol mannosides (PIMs), lipomannan (LM), and lipoarabinomannan (LAM). The biosynthesis of these lipids is believed to be prime drug targets, and has been clarified in great detail over the past several years.

Scope of Review: We will provide the current understanding of the inositol lipid metabolism in mycobacteria. We will highlight unsolved issues and future directions especially in the context of metabolic regulation.

Major Conclusions: Inositol is a building block of phosphatidylinositol (PI), which is further elaborated to become PIMs, LM and LAM. D-*myo*-inositol 3-phosphate is an intermediate of the *de novo* inositol synthesis, but it is also the starting substrate for mycothiol synthesis. Therefore, controlling the level of D-*myo*-inositol 3-phosphate is probably important to maintain the balance between mycothiol and inositol lipids. Several additional control mechanisms must exist to control the complex biosynthetic pathways of PI, PIMs, LM and LAM. The control mechanisms likely include regulatory proteins such as a lipoprotein LpqW, and spatial dynamics of enzymes as seen in the amphipathic property of PimA and membrane compartmentalization of PIM/LM biosynthetic enzymes. Furthermore, producing properly sized LM/LAM appear to require the control of glycan polymer elongation.

General Significance: Mycobacteria have evolved a highly sophisticated network of inositol metabolism. Clarifying its metabolism will not only provide better understanding of bacterial pathogenesis, but also understanding of the evolution and general functions of inositol lipids in nature.

2. Morita YS, Yamaryo-Botte Y, Miyanagi K, Callaghan JM, Patterson JH, Crellin PK, Coppel RL, Billman-Jacobe H, Kinoshita T, McConville MJ. (2010)

#### **Stress-induced synthesis of phosphatidylinositol 3-phosphate in mycobacteria.**

J Biol Chem, 285: 16643-16650.

Phosphoinositides play key roles in regulating membrane dynamics and intracellular signaling in eukaryotic cells. However, comparable lipid-based signaling pathways have not been identified in bacteria. Here we show that Mycobacterium smegmatis and other Actinomycetes bacteria can synthesize the phosphoinositide, phosphatidylinositol 3-phosphate (PI3P). This lipid was transiently labeled with [(3)H]inositol. Sensitivity of the purified lipid to alkaline phosphatase, headgroup analysis by high-pressure liquid chromatography, and mass spectrometry demonstrated that it had the structure 1,2-[tuberculostearoyl, octadecenoyl]-sn-glycero 3-phosphoinositol 3-phosphate. Synthesis of PI3P was elevated by salt stress but not by exposure to high concentrations of non-ionic solutes. Synthesis of PI3P in a cell-free system was stimulated by the synthesis of CDP-diacylglycerol, a lipid substrate for phosphatidylinositol (PI) biosynthesis, suggesting that efficient cell-free PI3P synthesis is dependent on *de novo* PI synthesis. In vitro experiments further indicated that the rapid turnover of this lipid was mediated, at least in part, by a vanadate-sensitive phosphatase. This is the first example of *de novo* synthesis of PI3P in bacteria, and the transient synthesis in response to environmental stimuli suggests that some bacteria may have evolved similar lipid-mediated signaling pathways to those observed in eukaryotic cells.

3. Sena CB, Fukuda T, Miyanagi K, Matsumoto S, Kobayashi K, Murakami Y, Maeda Y, Kinoshita T, Morita YS. (2010)

#### **Controlled expression of branch-forming mannosyltransferase is critical for mycobacterial lipoarabinomannan biosynthesis.**

J Biol Chem, 285: 13326-13336.

Lipomannan (LM) and lipoarabinomannan (LAM) are phosphatidylinositol-anchored glycans present in the mycobacterial cell wall. In *Mycobacterium smegmatis*, the mannan core of LM/LAM constitutes a linear chain of 20-25 alpha1,6-mannoses elaborated by 8-9 alpha1,2-monomannose side branches. At least two alpha1,6-mannosyltransferases mediate the linear mannanose chain elongation, and one branching alpha1,2-mannosyltransferase (encoded by MSMEG\_4247) transfers monomannose branches. An MSMEG\_4247 deletion mutant accumulates branchless LAM and interestingly fails to accumulate LM, suggesting an unexpected role of mannanose branching for LM synthesis or maintenance. To understand the roles of MSMEG\_4247-mediated branching more clearly, we analyzed the MSMEG\_4247 deletion mutant in detail. Our study showed that the deletion mutant restored the synthesis of wild-type LM and LAM upon the expression of MSMEG\_4247 at wild-type levels. In striking contrast, overexpression of MSMEG\_4247 resulted in the accumulation of dwarfed LM/LAM, although monomannose branching was restored. The dwarfed LAM carried a mannan chain less than half the length of wild-type LAM and was elaborated by an arabinan that was about 4 times smaller. Induced overexpression of an elongating alpha1,6-mannosyltransferase competed with the overexpressed branching enzyme, alleviating the dwarfing effect of the branching enzyme. In wild-type cells, LM and LAM decreased in quantity in the stationary phase, and the expression levels of branching and elongating mannosyltransferases were reduced in concert, presumably to avoid producing abnormal LM/LAM. These data suggest that the coordinated expressions of branching and elongating mannosyltransferases are critical for mannan backbone elongation.

4. Kataoka K, Fujihashi K, Oma K, Fukuyama Y, Hollingshead SK, Sekine S, Kawabata S, Ito HO, Briles DE, Oishi K. (2011)

**Nasal Dendritic Cell Targeting Flt3 Ligand As A Safe Adjuvant Elicits Effective Protection Against Fatal Pneumococcal Pneumonia.**

*Infect Immun*, 2011 May 2. [Epub ahead of print]

We have previously shown that pneumococcal surface protein A (PspA)-based vaccine containing DNA plasmid encoding the Flt3 ligand (FL) gene (pFL) as nasal adjuvant prevented nasal carriage of *Streptococcus pneumoniae*. In this study, we further investigated the safety and efficacy of this nasal vaccine for the induction of PspA-specific antibody (Ab) responses against lung infection with *S. pneumoniae*. C57BL/6 mice were nasally immunized with recombinant PspA/Rx1 (rPspA) plus pFL three times at weekly intervals. When dynamic translocation of pFL was initially examined, nasal pFL was taken-up by nasal dendritic cells (DCs) and epithelial cells (nECs), but not in the central nervous systems including olfactory nerve and epithelium. Of importance, nasal pFL induced FL protein synthesis with minimum levels of inflammatory cytokines in the nasal washes (NWs) and bronchoalveolar lavage fluid (BALF). NWs and BALF as well as plasma of mice given nasal rPspA plus pFL contained increased levels of rPspA-specific secretory-IgA and IgG Ab responses that are correlated with elevated numbers of CD8(+) and CD11b(+) DCs and IL-2 and IL-4 producing CD4(+) T cells in the nasopharyngeal-associated lymphoid tissues (NALT) and cervical lymph nodes (CLNs). The *in vivo* protection by rPspA-specific Abs was evident by reduced numbers of CFU in the lungs, airway secretions, and blood when mice were nasally challenged with *Streptococcus pneumoniae* WU2. Our findings show that nasal pFL is a safe and effective mucosal adjuvant for the enhancement of bacterial Ag (rPspA)-specific protective immunity through DC-induced Th2-type and IL-2 cytokine responses.

5. Ezoe H, Akeda Y, Piao Z, Aoshi T, Koyama S, Tanimoto T, Ishii KJ, Oishi K. (2011)

**Intranasal vaccination with pneumococcal surface protein A plus poly(I:C) protects against secondary pneumococcal pneumonia in mice.**

*Vaccine*, 29:1754-1761.

Effective pneumococcal vaccines are required for preventing secondary bacterial pneumonia, a life-threatening condition, during epidemics of influenza. We examined whether nasal administration of a low dose of pneumococcal surface protein A (PspA) plus polyinosinic-polycytidylic acid (poly(I:C)) could protect against a fatal secondary pneumococcal pneumonia after influenza A virus infection in mice. PspA-specific IgG but not IgA level was higher in the airways and blood of mice nasally administered a low dose of PspA plus poly(I:C) than in mice nasally administered PspA alone or poly(I:C) alone. Binding of PspA-specific IgG increased C3 deposition on the bacterial surface. The survival rate during secondary infection was higher in mice immunized with PspA plus poly(I:C) than in mice immunized with poly(I:C) alone. The significant reduction in bacterial density in the lung and blood was associated with increased survival of immunized mice with secondary pneumonia. Passive transfer of sera from mice immunized with PspA plus poly(I:C) increased the survival of mice infected with secondary pneumonia. Our data suggest that an intranasal PspA vaccine has promising protective effects against secondary pneumonia after influenza and that PspA-specific IgG plays a critical role in this protection.

