# 論文内容の要旨

## 論文題目

Critical roles of M cell and gut-associated lymphoid tissue (GALT) in the induction of antigen-specific IgA immune response

(特異的 IgA 産生における M 細胞と腸管関連リンパ組織の重要性)


## ジェミウソン　ソアレス　ポンテス

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The mucosal surfaces are the biggest part of immune system represented by an ample area of tissues immunologically active that is covered by fine epithelial barriers. As the majority of infections are initiated at mucosal sites, the mucosal immune system is considered to be crucial line of defense against infectious disease. Despite the fact that the mucosal immune system is constituted for anatomical and functionally distinct compartments located far way from each other, the oral ingestion of antigen induces humoral and cellular responses not strictly to the site of antigen introduction but in the other mucosal compartments as well. In the gastrointestinal tract (GI), the episodes responsible for the induction of most of antigen-specific responses take place at specific sites in the mucosa that are characterized by the presence of organized lymphoid tissues. These tissues are consisted of lymphoid follicles such as Peyer's patch (PP) and mesenteric lymph node (MLN), which are constituted basically by antigen-presenting cells (APCs), immature B cells and adjacent T cells. Antigens in general are effectively taken up from the lumen in the specialized region called follicle-associated epithelium (FAE), located in the PP, structure

considered to be crucial for the induction of Ag-specific mucosal immune response. The FAE localizes such uptake to sites where antigens and pathogens can be efficiently processed and presented for induction of appropriate immune response. This process named as transcytosis is performed by specialized cells called microfold cells (M cells). However, the molecular mechanism involved in the uptake and trancytosis of luminal antigen and microbes by M cells is not clear. In this study, I evaluated the efficiency of mucosal vaccine targeting to M cells via specific transcytotic receptors and the roles of gut-associated lymphoid tissue (GALT) in the induction of mucosal immune response through M cell targeted antigen delivery. Firstly, the results demonstrated that the glycoprotein 2 (GP2) specifically expressed on the apical plasma membrane of M cells serves as a transcytotic receptor for mucosal antigens. GP2 protein selectively bound to enteropathogenic bacteria *Salmonella enterica* serovar *typhimurium*, by recognizing FimH-expressing type 1 pili on the bacterial outer membrane and induced efficient bacteria translocation from lumen to adjacent lymphoid tissues. However, M cells in $Gp2^{-/-}$ mice displayed defect in transcytosis of type1-piliated bacteria by M cells, resulting in attenuation of immune response in PPs. Likewise, both mucosal and systemic immune responses against tetanus toxoid (TT) expressed by recombinant S. *typhimurium* were efficiently induced in $Gp2^{+/+}$ mice, but not $Gp2^{-/-}$ mice, after oral administration. Thus, it is possible assume that GP2 is a novel transcytotic receptor on M cells for type I piliated bacteria that is prerequisite for mucosal immunosurveillance. These findings can be taken as a cornerstone to define the molecular basis of antigen transport by M cells. As M cell

demonstrated to be extremely efficient in transporting living particulate antigen, next, I assess the efficacy of soluble antigen delivery through PP and Villous M cells and the functions of GALT in the induction of mucosal immune response. By taking advantage of our previously established monoclonal antibody (NKM 16-2-4) that recognizes specifically murine M cells located in the epithelium of both Peyer's patches (PP M cells) and intestinal villi (villous M cells), via the recognition of M cell specific carbohydrate moiety containing α (1,2) fucose, PP-, MLN-, and PP and MLN double-deficient, and WT mice were orally immunized with TT conjugated with NKM 16-2-4 (NKM 16-2-4-TT) or control antibody (Rat IgG-TT). High levels of TT-specific serum IgG antibody responses were induced in all animals by oral administration with NKM 16-2-4-TT but not Rat IgG-TT. However, TT-specific intestinal IgA antibody responses were not induced in the PP- and double- deficient mice. Low levels of antigen-specific response were induced in the MLN-lacking mice compared to WT mice, but it was not statistically significant. These results suggest that PP is essential in the induction of Ag-specific mucosal IgA response after oral immunization with NKM 16-2-4 –TT. As the fucosylation in PP M cells and villous M cells is distinctly regulated by α (1,2) fucosyltransferases FUT1 and FUT2, respectively, FUT2-deficient mice were immunized with the complex NKM 16-2-4-TT in order to clarify the role of the uptake via villous M cells in the induction of mucosal immune response. All FUT2-deficient mice induced nice levels of TT-specific IgA antibody responses, but failed or induced low levels of TT- specific IgG response. These results highlight the importance of vaccine-delivery to PP and villous M cells in

promoting the induction of antigen-specific mucosal and systemic immune responses. Taken all together, the results provided for this study demonstrated that: (1) GP2-dependent transcytotic pathway would provide a new strategy for development of M-cell-targeted vaccines; (2) the PP is crucial for induction and regulation of Ag-specific IgA mucosal response against soluble antigen orally delivered via α (1,2) fucose moiety; and, finally, (3) the antigen-delivery to both PP and villous M cells might be important strategy for generation of antigen-specific mucosal immunity.