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論　文　内　容　の　要　旨

氏　名　（　劉　子莱　）	
論文題名	Extended application of *Alcaligenes* lipid A as a vaccine adjuvant （アルカリゲネスリピドAのワクチンアジュバントとしての応用展開）

論文内容の要旨

Research from ours and other laboratories previously identified *Alcaligenes* spp. as a commensal bacterium that resides in lymphoid tissues, including Peyer's patches. We found that *Alcaligenes*-derived lipopolysaccharide acted as a weak agonist of Toll-like receptor 4 due to the unique structure of lipid A, which lies in the core of lipopolysaccharide. This feature allowed the use of chemically synthesized *Alcaligenes* lipid A as a safe synthetic vaccine adjuvant that induces Th17 polarization to enhance systemic IgG and respiratory IgA responses to T-cell-dependent antigens (e.g., ovalbumin and pneumococcal surface protein A) without excessive inflammation. Here, I conducted two investigations aimed at expanding the adjuvant functionality of *Alcaligenes* lipid A.

The first investigation focused on its adjuvant effect on T-cell-independent antigens, which has not been previously examined. For this purpose, I examined the adjuvant activity of *Alcaligenes* lipid A on a *Haemophilus influenzae* B conjugate vaccine that contains capsular polysaccharide polyribosyl ribitol phosphate (PRP), a T-cell-independent antigen, conjugated with the T-cell-dependent tetanus toxoid (TT) antigen (i.e., PRP-TT). When mice were subcutaneously immunized with PRP alone or mixed with TT, *Alcaligenes* lipid A did not affect PRP-specific IgG production. In contrast, PRP-specific serum IgG responses were enhanced when mice were immunized with PRP-TT, but these responses were impaired in similarly immunized T-cell-deficient nude mice. Furthermore, TT-specific-but not PRP-specific-T-cell activation occurred in mice immunized with PRP-TT together with *Alcaligenes* lipid A. In addition, coculture with *Alcaligenes* lipid A promoted significant proliferation of and enhanced antibody production by B cells. Together, these findings suggest that *Alcaligenes* lipid A exerts an adjuvant activity on thymus-independent Hib polysaccharide antigen in the presence of a T-cell-dependent conjugate carrier antigen.

Second purpose of my study is to verify the application of *Alcaligenes* lipid A as a mucosal vaccine adjuvant. Mucosal vaccination is an ideal method to induce protective immunity against various pathogens. However, antigens alone are insufficient to elicit robust mucosal immune responses, necessitating the development of effective adjuvants. In this study, I also evaluated the effectiveness of *Alcaligenes*-derived lipid A as an adjuvant for sublingual immunization, a novel vaccination route garnering significant attention. Comparing to nasal administration as we previously examined, sublingual administration is able to not only induce immune responses in respiratory tracts but also in intestinal tracts with fewer safety issues. When mice were sublingually immunized with *Alcaligenes* lipid A and ovalbumin (OVA), a model antigen, an enhanced production of OVA-specific IgA was detected in both the respiratory and intestinal tracts, along with increased OVA-specific IgA and IgG antibodies in serum. Additionally, sublingual immunization with cholera toxin B subunit (CTB) and lipid A resulted in elevated levels of CTB-specific IgG and IgA responses in the intestinal tract and systemic compartments, leading to the suppression of diarrhea induced by oral challenge with cholera toxin. Furthermore, immunization with pneumococcal surface protein A (PspA) plus *Alcaligenes* lipid A induced high levels of PspA-specific Th17 responses, as well as IgA and IgG responses, in both the respiratory tract and systemic compartments, providing protection against *Streptococcus pneumoniae*

infection. These findings suggest that *Alcaligenes*-derived lipid A is a potent sublingual vaccine adjuvant with potential efficacy against both respiratory and intestinal infectious diseases.

As a conclusion, *Alcaligenes* lipid A could enhance immune responses against both T cell-dependent antigens and T cell-independent antigens and furtherly exert a role as a suitable sublingual vaccine adjuvant to help protect infection pathogens.

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論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

本論文においては、リンパ組織に常在する共生細菌であるAlcaligenes 菌に着目し、リポ多糖（LPS）に含まれるリピドAのアジュバント活性について、T細胞非依存性抗原であるインフルエンザ菌b型（Hib）莢膜多糖ポリリボシルリビトールリン酸（PRP）と、T細胞依存性抗原である破傷風トキソイド（TT）を結合させたHib結合ワクチン（PRP-TT）に対して検討した。マウスを皮下免疫した結果、PRP単独またはTTと混合した場合、AlcaligenesリピドAはPRP特異的IgG産生に影響を与えなかった。しかし、PRP-TTで免疫した場合には、PRP特異的血清IgG応答が増強され、この応答はT細胞欠損ヌードマウスでは顕著に低下した。さらに、PRP-TTとAlcaligenesリピドAを同時投与したマウスでは、TT特異的なT細胞活性化が確認されたが、PRP特異的T細胞活性化は認められなかった。また、B細胞との共培養実験では、AlcaligenesリピドAがB細胞の増殖を促進し、抗体産生を増強することが示された。

さらに投与経路の拡張として、舌下ワクチンへの拡張性を検討した。その結果、舌下免疫により呼吸器と腸管の両方に免疫応答が誘導でき、それぞれの感染モデルに対しても防御効果を発揮することを示した。

本研究の成果は、新規ワクチンアジュバントの開発に貢献する重要な知見であり、その学術的価値と研究の独創性を高く評価し、博士（薬科学）の学位論文に値するものと認める。