第 11 回次世代アジュバント研究会

11th Meeting of the Japanese Vaccine Adjuvant Research Consortium

<同時開催>

2nd ISV Asia Vaccine and Immunotherapeutic Symposium

2018 年 1 月 23 日(火)/ January 23, 2018

千里ライフサイエンスセンター / Senri Life Science Center, Osaka, Japan

主 催：国立研究開発法人医薬基盤・健康・栄養研究所

次世代アジュバント研究会

AMED 創薬基盤推進研究事業「革新的技術に裏打ちされた有効かつ安全な次世代アジュバント開発」研究班 (研究代表者 国立研究開発法人医薬基盤･健康･栄養研究所 理事長 米田 悦啓)

共 催：日米医学協力研究会免疫専門部会

<Organizer>

◆National Institutes of Biomedical Innovation, Health and Nutrition (NIBIOHN)

◆Japanese Vaccine Adjuvant Research Consortium

◆Research Committe"Development of the next-generation adjuvants with innovative evaluation systems for safety and efficacy" supported by Japan Agency for Medical Research and Development (AMED) Grant

<Supporting organization>

◆U.S.-Japan Cooperative Medical Science Program Immunology Board

NIBIOHN 国立研究開発法人 医薬基盤･健康･栄養研究所 National Institutes of Biomedical Innovation, Health and Nutrition

CVAR ワクチン・アジュバント研究センター Center for Vaccine & Adjuvant Research

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第1土曜特集

近未来のワクチン
――開発研究の潮流と課題

企画 石井 健 医薬基盤・健康・栄養研究所 ワクチン・アジュバント研究センター

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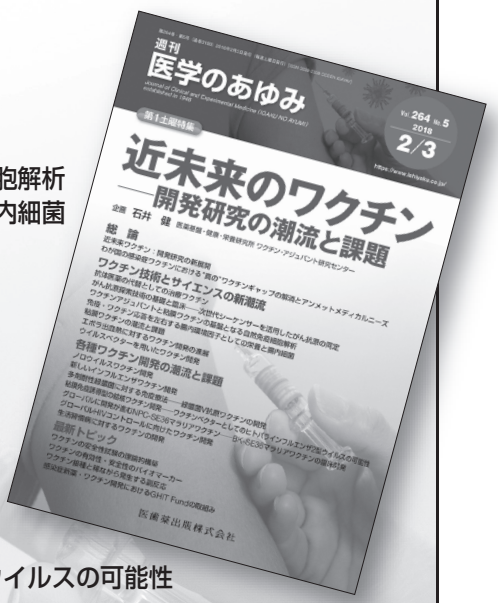

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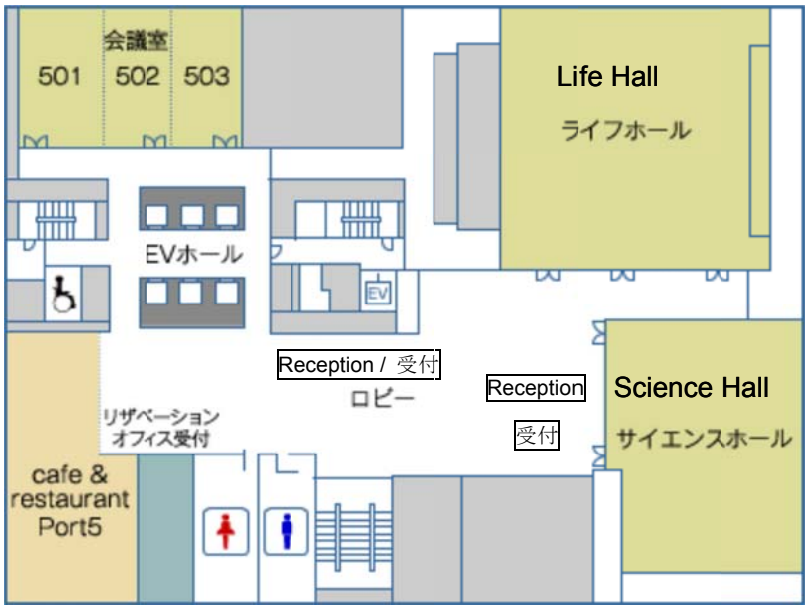
Venue / 会場



Venue for oral presentation
January 23 10:00-17:25

Venue for poster presentation
January 23 10:00-14:00

Notice / 注意

(1) Smoking is allowed only in the designated room on the 6th floor.
喫煙される方は、6 階の喫煙場所でお願いします。館内のそれ以外の場所での喫煙は禁じられています。

(2) Please turned off your cell phone or put them on vibrate during the presentations.
講演中は、携帯電話は電源を切るかマナーモードにしてください。

Program for oral presentation / 講演プログラム

■ **Registration (9:30)**

1. Asia Vaccine and Immunotherapeutic Symposium (10:00 ～ 12:10)

◆Opening remarks (10:00~10:05)

Margaret Ann Liu / International Society for Vaccine

◆Part 1（10:05 ~ 11:40）

＜session chair＞ Shan Lu / University of Massachusetts Medical School

Yasunori Yasutomi / NIBIOHN

【O-01】10:05~10:30

「Adjuvants improve the magnitude and durability of HIV vaccine-induced immune correlates」

Jerome H. Kim / International Vaccine Institute

【O-02】10:30~10:55

「Development of adjuvanted vaccines: from an empirical to an evidence-based approach」

Arnaud Didierlaurent / GlaxoSmithKline Vaccines, Belgium

【O-03】10:55~11:20

「Mucosal Vaccines for the Prevention of Aero-digestive Infection」

Hiroshi Kiyono / The University of Tokyo and Chiba University

【O-04】11:20~11:40

「Towards formulating an atherosclerosis vaccine」

Kouji Kobiyama / La Jolla Institute for Allergy and Immunology, USA

◆Part 2（11:40 ~ 12:10）

＜session chair＞ Joon Haeng Rhee / Chonnam National University

Ken J. Ishii / NIBIOHN

【O-05】11:40~11:55

「Squalene-Adjuvanted H7N9 Virus Vaccine Induces Robust Humoral Immune Response against H7N9 and H7N7 Viruses」

Juine-Ruey (JR) Chen / Adimmune Corporation, Taiwan

【O-06】11:55~12:10

「Intelligent emulsion functioned as potent adjuvant for prophylactic and therapeutic vaccinations」

Guanghui Ma / Chinese Academy of Sciences, China

2. Lunch Break（12:10 ~ 13:15）

3.Poster session & Coffee break (13:15 ～ 14:00)

◆Please feel free to come to Science Hall. Poster presenters will stand by their poster and be prepared to answer questions from attendees.

◆Please help yourself to some coffee at the lobby.

4. Meeting of the Japanese Vaccine Adjuvant Research Consortium (14:00 ～ 17:25)

◆Opening remarks (14:00~14:05)

Koichi Yamanishi / The research foundation for microbial disease of Osaka University

◆Part 1（14:05 ~ 15:20）

＜session chair＞ Yumiko Imai / NIBIOHN

【O-07】14:05~14:30

「Development of mycobacterial glycolipids-based adjuvants using nanoparticle technology」

Sho Yamasaki / Osaka University and Kyushu University

【O-08】14:30~14:55

「Intestinal dendritic cell and mucosal vaccine development」

Satoshi Uematsu / Chiba University and The University of Tokyo

【O-09】14:55~15:20

「Hydroxypropyl-β-Cyclodextrin, as a novel safe and effective adjuvant for seasonal influenza vaccine」

Etsushi Kuroda / National Institutes of Biomedical Innovation, Health and Nutrition (NIBIOHN) and Osaka University

◆Part 2（15:35 ~ 16:30）

＜session chair＞ Jun Kunisawa / NIBIOHN

【O-10】15:35 ~16:00

「Recognition of nanoparticles and crystals by scavenger receptors」

Masafumi Nakayama / Tohoku University

【O-11】16:00~16:15

「Construction of a cell-based assay system to develop high throughput screening system for vaccine adjuvant」

Eita Sasaki / National Institute of Infectious Diseases

【O-12】16:15~16:30

「Design, Synthesis and Biological Evaluation of Novel CD1d Ligands Containing Modified LipidMoieties」

Shinsuke Inuki / Keio University and Kyoto University

◆Part 3（16:30 ~ 17:20）

＜session chair＞ Ken J. Ishii / NIBIOHN

【O-13】16:30~16:55

「Possibilities of adjuvants use for allergen specific immunotherapy」

Katsuyo Ohashi-Doi / Torii Pharmaceutical Co. Ltd

【O-14】16:50~17:15

「Development of Therapeutic Vaccines for hypertension and diabetes」

Hironori Nakagami / Osaka University Graduate School of Medicine

◆ Closing remarks (17:20~17:25)

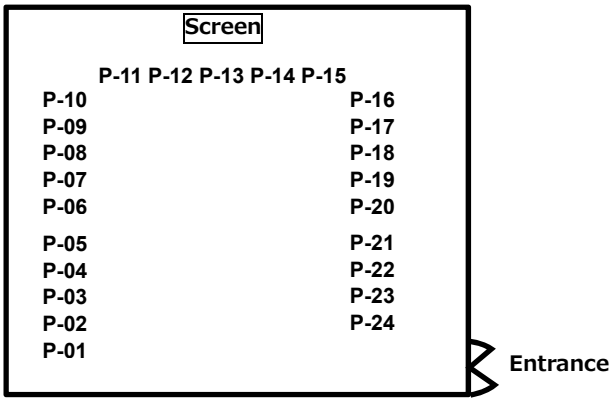
Yoshihiro Yoneda / National Institutes of Biomedical Innovation, Health and Nutrition (NIBIOHN)

Program for poster presentation / ポスター発表プログラム

【 January 23, 2018 (10:00 ～ 14:00)】

■ **Poster session : Poster presenters will stand by their poster and be prepared to answer questions from attendees from 13:15 to 14:00.**

＜Poster Layout ＞Science Hall



【P-01】

Development of Safe and Non-self Immunogenic Mucosal Adjuvant by Recombinant Fusion of Cholera Toxin A1 Subunit with Protein Transduction Domain

Man Ki Song / International Vaccine Institute,Republic of Korea

【P-02】

Harnessing Chaperna for the Assembly of Virus-like Particles as Recombinant Vaccines

Baik L. Seong / Yonsei University, Korea

【P-03】

Modification of HA glycosylation for better immunogenicity of avian influenza H7N9 vaccine

Man-Seong Park / Korea University College of Medicine, Republic of Korea,Man-Seong Park

【P-04】

Staphylococcal particulate cell wall releasing neutrophil-calprotectin has adjuvant activity as like aluminum salt

Bok Luel Lee / Pusan National University,South Korea

【P-05】

Neuropeptides control the pathology of severe influenza virus infection

Yumiko Imai / National Institute of Biomedical Innovation, Health and Nutrition (NIBIOHN)

【P-06】

Assessment of K3-SPG, nano-particulate CpG DNA, as an immunotherapeutic agent in non-human primate models in vivo

Yuji Masuta / National Institutes of Biomedical Innovation, Health and Nutrition (NIBIOHN), Osaka University and Nippon Shinyaku Co., Ltd

【P-07】
An approach to establish an in vitro assay system for the safety control of influenza vaccines and adjuvants
Haruka Momose / National Institute of Infectious Diseases
【P-08】
Enhancement of avidity and ADCC activities of virus-specific antibodies by TLR agonists improved vaccine efficacy of influenza split vaccines
Kayoko Sato /National Institute of Infectious Diseases
【P-09】
Serum bactericidal assay for the evaluation of typhoid vaccines using a semi-automated colony counting method
Jae Seung Yang / International Vaccine Institute, Republic of Korea
【P-10】
Relationship between the inducibility of the IFN-β production in Sendai virus Cantell strain and the antagonistic action toward the IFN-α/β signal transduction by the C protein
Kosuke Oda/ Graduate School of Biomedical and Health Sciences, Hiroshima University
【P-11】
Th17 promotes the induction of antigen-specific gut-mucosal cytotoxic T lymphocytes following intramuscular vaccination of an adenovirus vector
Masashi Tachibana / Osaka University
【P-12】
Organogenesis of inducible bronchus-associated lymphoid tissue plays an essential role in Ag85B-rhPIV2-based anti-tuberculosis respiratory vaccine in mice
Takahiro Nagatake / National Institutes of Biomedical Innovation, Health and Nutrition (NIBIOHN)
【P-13】
Prime-boost Vaccination with CpG ODN and curdlan strongly induces both systemic and mucosal immunity
Naoki Takemura / Chiba University and The University of Tokyo
【P-14】
T cell response analysis against human herpesvirus 6B glycoprotein complex vaccination
Mie Okutani / Osaka University
【P-15】
Cooperative Adjuvant Effect of Combination of 2-Hydroxypropyl-*β*-Cyclodextrin and CpG-ODN
Tomoya Hayashi / National Institutes of Biomedical Innovation, Health and Nutrition (NIBIOHN) and Kumamoto University
【P-16】
Adjuvant X can induce both cellular and humoral immunity even at very low antigen dose
Yasunari Haseda / Osaka University

【P-17】

Induction of IL-12p70 by a novel oligonucleotide in human PBMC

Jie Meng / Osaka University

【P-18】

Vaccine adjuvant effects of dendritic cell-targeting peptides

Kazuki Misato / Osaka University

【P-19】

Efficient induction of CD8+ cytotoxic T lymphocytes response by mutant dendritic cell-targeting peptide

Yuki Kanai/ Osaka University

【P-20】

Activation of CD4+ T cell response by using dendritic cell-targeting peptide

Seiki Shirai / Osaka University

【P-21】

Mechanism of synthetic hemozoin adjuvanticity

Michelle Sue Jann Lee/ Osaka University

【P-22】

ZBP1 governs neutrophil-mediated inflammation in influenza virus infection via IL-1a

Masatoshi Momota / National Institutes of Biomedical Innovation, Health and Nutrition (NIBIOHN) and Osaka University

【P-23】

The role of Interleukin-1a and DNA in the particles-associated inflammation

Kou Hioki / Osaka University

【P-24】

Immunological mechanism of synergistic anti-cancer activities by activation of TLR9 and STING

Burcu Temizoz / Osaka University and National Institute of Biomedical Innovation, Health and Nutrition (NBIOHN)

Abstract of oral presentation / 講演要旨 【O-01】

Adjuvants improve the magnitude and durability of HIV vaccine-induced immune correlates

Jerome H. Kim, MD

International Vaccine Institute

The RV144 HIV vaccine trial with ALVAC-HIV prime and gp120 B/E boost showed 31% protection from HIV acquisition at 42 months. Correlates analysis revealed that antibody to the V1V2 region of HIV-1 Env was associated with a decreased risk of infection whereas IgA to Env was associated directly with infection risk. anti-V1V2 responses were important in selection of gp120 subtype C antigens for efficacy trials in the Republic of South Africa that are now under way. Post-hoc analysis of simultaneous vaccine efficacy (VE) suggested that efficacy was 60% at month 12, and declined steadily thereafter. In light of these findings we looked to different approaches to improve the magnitude and durability of anti-V1V2 responses. Data suggested that a late dose of gp120 B/E – given more than 7 years after the primary series was associated with striking V1V2 responses, exceeding that seen in RV144. When tested formally, 12 and 15 month "booster" doses were associated with prolonged elevation of V1V2 antibody. Older unpublished data suggested that adding monophorphoryl lipid A liposomes to alum-adjuvanted gp120 resulted in significant increases in magnitude and durability of anti-V1V2 responses. In NHP, SIV challenge studies using SIV cognates of the vaccines from RV144 showed that alum adjuvanted gp120 as a boost to ALVAC-SIV was associated with the same level of protection seen in RV144, however, adjuvanting in MF59 resulted in no evidence of protection from SIV acquisition despite higher levels of antibody. These data are discussed in the context future trial design.

Abstract of oral presentation / 講演要旨　【O-02】

Development of adjuvanted vaccines: from an empirical to an evidence-based approach

Dr. Arnaud Didierlaurent

Senior Director, Head of adjuvant platform
GlaxoSmithKline Vaccines, Belgium

Adjuvants are now being used in licensed vaccines with proven clinical benefits and established safety. Several recent studies on their mode of action have provided insight into how adjuvants impact the immune response. Specific molecular and cellular signatures associated with improved immunogenicity of adjuvanted vaccines are now emerging from those studies, in particular from system vaccinology approaches conducted in humans or relevant animal models. This provides novel targets for the development of new generation adjuvants based on synthetic molecules and improved formulations. The presentation will provide examples on those immune signatures that are associated with improved potency and how they can be used to develop new adjuvants in the future.

Abstract of oral presentation / 講演要旨 【O-03】

Mucosal Vaccines for the Prevention of Aero-digestive Infection

Hiroshi Kiyono

Division of Mucosal Immunology
International Research and Development Center for Mucosal Vaccine
The Institute of Medical Science, The University of Tokyo

Department of Immunology, Graduate School of Medicine, Chiba University

Mucosal vaccines induce antigen-specific immune responses at mucosal sites including the respiratory, digestive, and reproductive tracts, and also inducing immunity in the systemic compartment. Especially nasal and oral vaccinations are effective mucosal immunization routes to prevent infectious pathogens invade the body through the respiratory and digestive mucosa. Moreover, it is also attractive method because the physical and psychological discomfort associated with injection methods could be eliminated. Based on the advantages, we have been developing novel nasal vaccination system using cationic cholesteryl group–bearing pullulan (cCHP) nanogels. Vaccine antigens incorporated into cCHP nanogels induced potent antigen-specific mucosal and systemic immune responses and have exhibited no brain deposition of antigen via olfactory bulb after nasal administration in mice and nonhuman primates. Thus, cCHP based nanogel is a safe and effective nasal vaccine delivery system. For the intestinal infectious disease, our efforts have been aiming the development of oral vaccine using the rice transgenic system, MucoRice expressing vaccine antigen or neutralizing antibody for the prevention and/or treatment of gut pathogen induced diarrheal disease. We will present and discuss our progress on our efforts for the development of mucosal vaccines against respiratory (e.g., *Streptococcus pneumonia*) and intestinal (e.g., *Vibrio cholerae*) infectious diseases.

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Abstract of oral presentation / 講演要旨 【O-04】

Towards formulating an atherosclerosis vaccine

Kouji Kobiyama, Takayuki Kimura, Holger Winkels, Klaus Ley

La Jolla Institute for Allergy and Immunology, USA

Atherosclerosis is an inflammatory disease of the arterial wall. Its sequelae including myocardial infarctions, strokes, and peripheral artery disease, are the leading cause of death worldwide. The development of an effective atherosclerosis vaccine is an alluring medical technology that has the potential to prevent atherosclerosis and atherosclerosis-dependent diseases. We recently showed that vaccination with immunogenic major histocompatibility complex class II (MHC-II)-restricted peptides of apolipoprotein B (ApoB)-100 - the major antigen in atherosclerosis - induced IL-10 by T cells and conferred atheroprotection. However, most studies so far used a clinically non-translatable adjuvant, complete and incomplete Freund's adjuvants (CFA and IFA).

Thus, to develop an atherosclerosis-specific vaccine we aim to find 1) an effective and clinically acceptable vaccine adjuvant and to identify 2) candidate epitopes of human ApoB-100. We found that immunization of atherosclerotic mice with an MHC-II-restricted mouse ApoB-100 peptide (P6) in Addavax effectively reduced atherosclerotic burden. Addavax is a squalene-based adjuvant similar to MF59, which is FDA approved and licensed in Europe. Mechanistically, mice immunized with P6 in Addavax induced IL-10 production without eliciting an antibody response. These data suggest that an antigen-specific antibody may not be required for atheroprotection by vaccination and highlights MF59 as a candidate adjuvant.

In an effort to prioritize candidate epitopes of human ApoB-100, we discovered an ApoB-100 peptide, P18, whose sequence is identical in mice and humans and which binds both mouse and human MHC-II. Immunization with this peptide conferred atheroprotection and was accompanied by expansion of P18-specific regulatory T cells (Tregs). Of note, P18-specific $CD4^+$ T cells were detectable by HLA-DR1/P18 peptide tetramer in human PBMCs. Furthermore, P18-specific $CD4^+$ T cells from subclinical cardiovascular disease patients exhibited a higher frequency of $FoxP3^+RORγt^+$ cells as compared to healthy donors, indicating that ApoB-specific Tregs lose their anti-inflammatory phenotype in cardiovascular disease.

Abstract of oral presentation / 講演要旨 【O-05】

Squalene-Adjuvanted H7N9 Virus Vaccine Induces Robust Humoral Immune Response against H7N9 and H7N7 Viruses

Dr. Juine-Ruey (JR) Chen
R&D Deputy Director, Adimmune Corporation, Taiwan.

Recent cases of avian influenza H7N9 have caused great concerns that virus may become transmittable between humans. It is imperative to develop an effective vaccine to fight against the pandemic potential of this H7N9 influenza virus to protect human from the disease. Our study aims to investigate an optimized formulation for the development of H7N9 vaccines. Various doses of H7N9 inactivated whole or split virus antigens (0.5, 1.5, or 3μg based on hemagglutinin content) combined with squalene based adjuvant (AddaVAX), aluminum hydroxide $Al(OH)_3$ or without adjuvant were evaluated for the efficacy of low immunogenic H7N9 vaccine in mice and ferret. With either H7N9 whole or split-virus based vaccines, AddaVAX-adjuvanted formulations were the most immunogenic in eliciting significant humoral immune response against H7N9 virus and exhibited strong cross-reactive response in hemagglutination inhibition (HAI) and viral-neutralization assays against H7N7 virus as well. In contrast, formulations with $Al(OH)_3$ or without adjuvant were less immunogenic and elicited lower titers of HAI and microneutralization assays against both viruses. Protection experiments demonstrated that the formulation of 0.004μg to 0.5μg of split virion vaccines with AddaVAX conferred full protection against viral challenge up to 100 LD_{50} of wild-type H7N9 virus. The reduction of virus titers in different respiratory tissues by split virion combined with AddaVAX also provide better protection from the virus challenge in ferrets than split virion combined with $Al(OH)_3$. Taken together, our study demonstrates that squalene based adjuvant can significantly enhance the protective efficacy of H7N9 virus vaccine and provides a useful strategy to confront the potential pandemic outbreaks of H7N9 virus.

Intelligent emulsion functioned as potent adjuvant for prophylactic and therapeutic vaccinations

Guanghui Ma*

State Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100190, PR China

*Email: ghma@ipe.ac.cn

There is an urgent need to develop potent adjuvant for both humoral and cellular responses. “Bio-mimicking” is considered as the golden role in the adjuvant design. Previous attempts have focused on mimic the sizes, shapes, charges and compositions of pathogens. However, it is neglected that these microbes are actually soft materials, which demonstrate the force-dependent deformation and antigen mobility during cellular encounter. In light of these concerns, we designed intelligent emulsion which was stabilized by PLGA nanoparticle instead of conventional surfactant. It was demonstrated that the emulsions pliability during cellular encounter increased the contact area, and the adsorbed antigens among nanoparticles experienced lateral mobility to intensively interact with the receptors on the antigen presenting cells. These features significantly promoted antigen uptake among the recruited immunecytes. As charge-reversal effect in lower pH environment of lysosomes, PLGA nanoparticle-stabilized emulsions presented positive charges to cause the destabilization of lysosomes and thus triggered cytosolic delivery of antigens. Compared with solid particles and conventional surfactant-stabilize emulsions, the intelligent emulsions enhanced activation of the draining lymph nodes, and potently stimulated both humoral and cellular adaptive responses. The novel adjuvant was further employed for prophylactic and therapeutic vaccinations, which increased the survival of mice upon lethal challenge of H1N1 viruses and B16/MUC1melanoma. Accordingly, the pliability and lateral mobility of antigen-loaded PLGA nanoparticle-stabilized emulsions may offer an effective, safe and broadly applicable strategy to enhance adaptive immunity against infections and diseases.

Keywords Pickering emulsion, adjuvant, tumor immunotherapy.

Abstract of oral presentation / 講演要旨　【O-07】

Development of mycobacterial glycolipids-based adjuvants using nanoparticle technology

結核菌糖脂質−レクチン受容体相互作用を活用した新規ナノ粒子アジュバントの創成

Sho Yamasaki, Ph.D. / 山崎　晶

1 Department of Molecular Immunology, Research Institute for Microbial Diseases, Osaka University / 大阪大学　微生物病研究所　分子免疫制御分野

2 Department of Molecular Immunology, Immunology Frontier Research Center (IFReC), Osaka University / 大阪大学 免疫学フロンティア研究センター 分子免疫学

3 Division of Molecular Immunology, Medical Institute of Bioregulation, Kyushu University / 九州大学　生体防御医学研究所　免疫制御学分野

Rational design of adjuvants and delivery systems will promote development of next-generation vaccines to control emerging and re-emerging diseases. To accomplish this purpose, understanding the immune enhancing properties of new adjuvants relative to those induced by natural infections can help with the development of pathogen mimicking materials that will effectively initiate innate immune signaling cascades.

Mycobacterium tuberculosis, a causative agent of tuberculosis, has been known to possess potent adjuvant activities. Freund's complete adjuvant (CFA), which efficiently stimulates cell-mediated immunity, is composed of heat-killed *M. tuberculosis*. However, the precise molecular mechanisms by which *M. tuberculosis* exerts adjuvanticity have not been clearly understood for a few decades.

Among pattern recognition receptors (PRR) for pathogen-associated molecular patterns (PAMPs), C-type lectin receptors (CLRs) comprise a large family of proteins that share a common structural motif and are involved in various immune responses. We found that three ITAM-coupled CLRs, Mincle (Clec4e), MCL (Clec4d), Dectin-2 (Clec4n) and DCAR (Clec4b1), act as pattern recognition receptors (PRRs) for mycobacteria. Characteristic mycobacterial glycolipids, such as trehalose dimycolate (TDM) and lipoarabinomannan (LAM) and phopshatidylinositolmannoside (PIM), were identified as ligands for these receptors (Yamasaki, ***Nat. Immunol.*** 2008; Ishikawa, ***J. Exp. Med.*** 2009; Miyake, ***Immunity*** 2013; Yonekawa, ***Immunity*** 2014; Toyonaga, ***Immunity*** 2016). These findings shed light on CLRs as emerging immune receptor family for glycolipids derived from pathogens, particularly mycobacteria, and thus CLRs could be attractive targets for the development of novel adjuvants. To administrate these "water-insoluble" glycolipids *in vivo*, we hypothesized that recently developed nanoparticles could be useful as effective and safe delivery systems.

In this symposium, we will introduce our approach to develop novel adjuvant by decorating the surfaces of nanoparticles with those immunostimulatory glycolipids to confer "pathogen-like" properties and enhance adjuvanticity.

Abstract of oral presentation / 講演要旨 【O-08】

Intestinal dendritic cell and mucosal vaccine development

Satoshi Uematsu, M.D., Ph.D.

[1] Professor Department of Mucosal Immunology, Graduate School of Medicine, Chiba University Project Professor

[2] Division of Innate Immune Regulation, International Research and Development Center for Mucosal Vaccines, Institute of Medical Science, The University of Tokyo

Vaccines are effective prophylaxis against infectious diseases. Conventionally, vaccine antigens (Ag) are injected intramuscularly or subcutaneously to induce Ag-specific IgG and Th1 responses, which prevent disease exacerbations through elimination of invading pathogens and infected cells. However, injectable vaccines poorly induce mucosal immunity, such as Ag-specific secretary (s)IgA and Th17 responses, which exert barrier functions to block pathogen entry and colonization at mucosal surfaces. Because such responses are regulated by unique subset of dendritic cells (DCs) that reside only in mucosa, topical application of vaccine is necessary to deliver Ag to mucosal DCs for inducing Ag-specific mucosal immunity. However, the efficiency of mucosal vaccines in inducing systemic immunity is relatively low, because of physical disturbance of Ag delivery to mucosal DCs by epithelial barriers. Furthermore, immune tolerance induction is a big problem especially in gut. Co-administration of toxin-based adjuvants has been tested to overcome these difficulties but are unavailable for humans due to their toxic side effects. Thus, development of an innovative vaccination method which can induce both systemic and mucosal immunity adequately has been aspired. Our previous reports showed that $CD103^{+}CD11b^{+}$ DCs in small intestinal lamina propria (siLPDCs) induced intestinal mucosal immunity by Toll-like receptor ligands stimulation such as flagellin and CpG oligodeoxynucleotides (CpG-ODN). Unlike splenic(SP) DCs, they specifically expressed retinoic acid-converting enzyme retinal dehydrogenase isoform 2 (RALDH2) and could induce Ag-specific sIgA and Th17 responses in addition to IgG and Th1 responses. Here, we find that SPDCs co-stimulated by CpG-ODN and curdlan induce similar immune responses to those induced by activated siLPDCs. This adjuvant combination not only induces activation but also up-regulates the expression of RALDH2 and transforming growth factor-β in SPDCs, respectively, critical for IgA and Th17 inductions. Intramuscular Ag injection with CpG-ODN and curdlan induces Ag-specific systemic responses such as IgG and Th1. Surprisingly, the priming immunization also induces Ag-specific sIgA in stool, which protects cholera toxin-induced diarrhea. Although Ag-specific sIgA induction is transient after the priming, oral Ag administration induces high levels of Ag-specific IgA in stools for over 3 months. Moreover, Ag-specific Th1 and Th17 responses are induced in intestine after the boosting. Boosting effect is also induced in lung by intranasal Ag administration, thereby effectively inhibiting the colonization and invasion of *Streptococcus pneumoniae.* Collectively, this new prime-boost vaccination is the hybrid method of injectable and mucosal vaccines and will be an innovative technique to effectively prevent both invasion and disease progression of pathogens at the target mucosal tissues.

Abstract of oral presentation / 講演要旨　【O-09】

Hydroxypropyl-β-Cyclodextrin, as a novel safe and effective adjuvant for seasonal influenza vaccine.

Etsushi Kuroda[1,2], Takato Kusakabe[1,2], Shingo Kobari[1], Motoyasu Onishi[1] and Ken J. Ishii[1,2]

[1] Laboratory of Adjuvant Innovation, Center for Vaccine and Adjuvant Research (CVAR), National Institutes of Biomedical Innovation, Health and Nutrition (NIBIOHN)

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Adjuvants are often used to improve the effectiveness of vaccines for initiating the protective immunity. Although alum is the most widely used adjuvant in human vaccines, alum has certain limitations; low potency for cellular immune responses, IgE induction, formation of granuloma and long-term local inflammation at the site of injection. Therefore, there are needs for an alternative adjuvant with even better safety and efficacy than Alum.

Recently we found that hydroxypropyl-β-cyclodextrin (HP-β-CD), a widely used pharmaceutical excipient to variety of drugs, can function as potent adjuvant for Flu vaccines by enhancing antigen-specific IgG, but not IgE responses via similar mechanism of Alum adjuvant.[1] Furthermore, we also observed that HP-β-CD is applicable to mucosal vaccine adjuvant for the induction of antigen-specific mucosal immune responses by intranasal administration.[2] Since HP-β-CD is an approved pharmaceutical excipient, these findings indicate that HP-β-CD can be a safe and effective adjuvant for human vaccines.

In this presentation, we will talk about the mode of action of HP-β-CD and the application as a novel flu vaccine adjuvant.

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Recognition of nanoparticles and crystals by scavenger receptors
スカベンジャー受容体によるナノ粒子および結晶粒子の認識機構

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Nanoparticles and crystals such as silica, alum, and monosodium urate crystals cause inflammation, which is triggered by phagocyte NLRP3 inflammasome activation and cell death. While the molecular mechanisms underlying particle-induced NLRP3 inflammasome activation have been extensively studied, it is poorly understood how macrophages recognize these particles on their cell surface. Given the negatively charged surface, inorganic particles may be also recognized by receptors for organic particles such as bacteria in a charge-dependent manner. Indeed, the class A scavenger receptors such as SR-A1 and MARCO bind silica and titanium particles as well as bacteria; however, these receptor-deficient mice and macrophages have still shown inflammatory responses to these inorganic particles, suggesting that additional receptor(s) may be involved. Using unbiased functional screening, we have recently identified the class B scavenger receptor member 1 (SR-B1) as a novel silica receptor. Both mouse and human SR-B1-mediated tethering of silica to macrophage surface and the subsequent phagocytosis cause NLRP3 inflammasome activation and cell death. I here focus on the role of scavenger receptors in nanoparticles and crystal-induced inflammation.

Construction of a cell-based assay system to develop high throughput screening system for vaccine adjuvant
ワクチンアジュバントのハイスループットスクリーニング系構築を目指した培養細胞による *in vitro* 評価系の構築

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In recent years, adjuvanted vaccines have been licensed and developed in order to increase the effectiveness of the vaccine. Since the mechanism of action of adjuvants are diverse, development studies are vigorously undertaken to develop and explore more effective and safety adjuvants. In recent years, by rapidly evaluating the efficacy and safety of candidate compounds at the early stages of drug development by utilizing high throughput screening (HTS), shorten development time and efficient strategies for seeds screening has been possible. However, in vaccine's adjuvants, such a HTS applicable for adjuvants has not been existed, and efficiency in development is considered to be lower than that of synthetic drugs. Thus, we have attempted to construct an *in vitro* HTS system useful for evaluating the efficacy and safety of vaccine adjuvant. Previously, we identified the lung biomarker genes useful for evaluating the bioactivity of vaccines and adjuvants. Among the biomarker genes, the genes have been considered involving "effectiveness" (infection prevention) and "toxicity" of vaccines. This lung biomarker genes is also expressed in some cultured cell lines, therefore construction of an HTS system using cultured cells is expected. Here we present an attempt to construct a vaccine and adjuvant biological activity evaluation method using the biomarker genes, alveolar epithelium/bronchial epithelial cell lines and human peripheral blood mononuclear cells (PBMC). Regarding alveolar epithelium/bronchial epithelial cell lines, increased expression levels of the biomarker genes and cytokines secretions response to inactivated influenza vaccine stimulation were observed. These reactions were strongly observed in a whole-virion inactivated influenza vaccine (WPV), which has high reactogenicity in humans. On the other hand, no responses to adjuvant supplementation such as toll-like receptor (TLR) agonist were observed. In human PBMC, WPV stimulation-dependent increases of the biomarker genes and cytokines secretions were observed. Notable cell death and cell proliferations by vaccine stimulations were not observed in T cells, B cells, NK cells and dendritic cells (DC). Furthermore, when PBMC enriched with plasmacytoid DC (pDC) was used, sensitivities to biomarker genes expressions against WPV stimulation were improved, suggesting that the biological activities assessed by the marker genes in PBMC would captures the reaction derived from pDC.

Design, Synthesis and Biological Evaluation of Novel CD1d Ligands Containing Modified Lipid Moieties
脂質部位の構造展開を基盤とする新規 CD1d リガンドの創製研究

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井貫晋輔[1,2]，平田菜摘[1]，柏原瑛美[1]，岸惇一郎[1]，藤本ゆかり[1]

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CD1d is a non-polymorphic MHC class I-like molecule, whose ligands include glycolipids such as α-GalCer (KRN7000).[1)] Complexes of glycolipid ligands and CD1d can be recognized by T cell receptors (TCR) found on natural killer (NK) T cells and thereby induce the secretion of various cytokines, including Th1 and Th2 cytokines.[2)] The control over the cytokine levels and balance is important for the development of effective immunotherapies. Thus, in an effort to identify novel immunomodulators, several structure-activity relationship (SAR) studies of CD1d ligands have been conducted to date.[3)] Intriguingly, these SAR studies revealed that the production and balance of cytokine levels are dependent on CD1d ligand structures.[3)]

The CD1d contains a large hydrophobic lipid binding pocket: the A' pocket of CD1d, which recognizes hydrophobic moieties of the ligands, such as long fatty acyl chains. The A' pocket is a large hydrophobic lipid binding groove surrounded by apolar amino acid residues. However, the pocket includes a few polar amino acid residues (eg. Ser28). We focused on the polar residues in the A' pocket of CD1d, and designed CD1d ligands that can interact with the hydrophilic residues through hydrogen bonds. A series of the ligands, α-GalCer derivatives containing polar groups in the acyl chain, was synthesized, and the structure-activity relationship studies demonstrated that simple modification from a methylene to an amide group in the long fatty acyl chain, when introduced at optimal positions, enhanced the CD1d recognition of the glycolipid ligands. Taken together, we demonstrated that confined polar residues in the large hydrophobic area of the lipid binding pockets of CD1d could be targeted to significantly influence the affinity between the protein and its ligands. These findings could provide a practical guide to designing lipid ligands and expanding opportunities to utilize these ligands as pharmaceuticals and adjuvants.[4)]

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Possibilities of adjuvants use for allergen specific immunotherapy

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Allergen specific immunotherapy (AIT) has been used for more than 100 years as a desensitizing therapy for IgE-mediated allergic diseases and represents the only potentially curative way of treatment. AIT is currently administered via either the subcutaneous (SCIT: subcutaneous immunotherapy) or sublingual (SLIT: sublingual immunotherapy) route and is recognized as the only treatment option with the potential to provide long-term post-treatment benefits and alter the natural course of allergic disease. The prevalence of Japanese cedar pollinosis in Japan is very high as 36.7% of the populations suffer from clinical allergy and 56.3% are sensitized with Japanese cedar pollen-specific IgE. However, even though SCIT has been available in Japan for many years it is not widely practiced thus far because of the disadvantages of SCIT, such as the need for frequent hospital visit, pain caused by injections, and the risk of systemic reactions. Therefore most of the patients have been treated with pharmacotherapy such as anti-histamine tablets. In 2014, a new treatment option with the introduction of SLIT-drop product for Japanese cedar pollinosis, a new treatment option for Japanese cedar pollinosis was presented, and a HDM SLIT-tablet for HDM-induced allergic rhinitis was approved in 2015. Both are based on SLIT and do not have the same disadvantages as SCIT.

The goal of successful AIT is to relieve symptoms by modulating the immunological mechanisms related to allergy. Our current understanding of the AIT mechanism of action emphasizes a prominent role for CD4+ cells in controlling effector immune mechanisms linked with allergic inflammation. Particularly, most allergic patients exhibit allergen-specific Th2 responses, and CD4+ T cell responses are redirected toward the Th1 type with an increased production of IFNγ as well as towards IL-10 producing regulatory T cells. Tolerance induction also requires allergen-specific regulatory B cells as well as a decreases in allergen-specific IgE levels over time followed by increasing of blocking antibodies levels (i.e., IgG and IgA). Although AIT has proven to be clinically effective and the more convenient SLIT-tablets were developed, AIT remains underused and is estimated to be used in less than 10% of allergic patients in worldwide. This is mainly due to several important drawbacks, including safety concerns or requirement for long term treatment and low patient adherence. Therefore, benefit of adjuvant in AIT has received much attention in this field, in order to help lowering the allergen dose, improving the safety profile with less local reactions and maintain or increase robust immunomodulation. In my presentation, the benefit of adjuvant use for next generation of AIT drug development will be discussed.

Abstract of oral presentation / 講演要旨 【O-14】

Development of Therapeutic Vaccines for hypertension and diabetes

生活習慣病を標的とした治療ワクチンの開発

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Recent research on vaccination has extended its scope from infectious diseases to chronic diseases, including Alzheimer's disease, hypertension and diabetes. We have recently reported that angiotensin II vaccine for hypertension or DPP4 vaccine for diabetes successfully attenuated the high blood pressure or hyperglycemia in each mice model, respectively (PNAS 2014, Hypertension 2015, Sci Rep 2017). The immunogenic molecule (i.e. KLH) is used to provide an antigen that supports the activation of helper T-cells in this vaccine system with co-treatment of Freund's adjuvant. Anti-angiotensin II antibodies has been induced by two or three times injection of vaccine at two weeks intervals and sustained for several months, which can efficiently ameliorate angiotensin II-induced high blood pressure and perivascular fibrosis in mice. We have also attempted to develop a therapeutic vaccine against DPP4. As a result, the vaccine-induced anti-DPP4 antibodies can efficiently ameliorate hyperglycemia in diabetic mice without any immunogenic side effects. From the clinical point of view, increasing the effectiveness of drug adherence interventions may have a great impact on the health of the population, because it is reported that approximately 50% may not take medications among patients with chronic illness. This poor adherence to medication leads to increased morbidity and death. If the improvement of drug compliance has been achieved with vaccines in hypertensive patients, it may assist better control of blood pressure, leading to reduced complications. We are now going to apply the therapeutic vaccine for another chronic diseases under the collaboration with the physician-scientists for translational research. The potential of a therapeutic vaccine will give us a chance to challenge to develop an innovative treatment for incurable diseases.

Abstract of poster presentation / 発表要旨 【P-01】

Development of Safe and Non-self Immunogenic Mucosal Adjuvant by Recombinant Fusion of Cholera Toxin A1 Subunit with Protein Transduction Domain

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Abstract

Potential use of cholera toxin (CT) as a mucosal vaccine adjuvant has been documented in a variety of animal models. However, native CT is highly toxic to be used as a mucosal adjuvant in humans. Here, we demonstrate a new approach to generate a mucosal adjuvant by replacing the B subunit of CT with HIV-1 Tat protein transduction domain (PTD), which efficiently delivers fusion proteins into the cell cytoplasm by unspecific binding to cell surface. We compared the adjuvanticity and toxicity of Tat PTD-CTA1-Tat PTD (TCTA1T) with that of CT. Our results indicate that intranasal (i.n.) delivery of ovalbumin (OVA) with TCTA1T significantly augments the OVA-specific systemic and mucosal antibody responses to levels comparable to those seen with CT adjuvant. Moreover, in vivo cytotoxic T lymphocyte activity elicited by TCTA1T was significantly higher than that elicited by a mutant TCTA1T (TmCTA1T) lacking ADP ribosyltransferase function. In addition, co-administration of influenza M2 protein with TCTA1T conferred near complete protection against lethal influenza virus challenge. Importantly, TCTA1T, in contrast to CT, did not induce serum IgG antibody responses to itself and was shown to be non-toxic. These results suggest that TCTA1T may be a safe and effective adjuvant when given by mucosal routes.

Abstract of poster presentation / 発表要旨 【P-02】

Harnessing Chaperna for the Assembly of Virus-like Particles as Recombinant Vaccines

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Significant advances have been made in the design and manufacture of nanoparticle vaccine, as vital alternative to traditional cell-culture vaccines. Assemblages of key immunologic features of viruses as highly repetitive particulate structures are essential for inducing potent and long-lasting antibody responses. The folding of monomeric antigens and their subsequent assembly into higher ordered structure is crucially important for robust and faithful production in a timely and reproducible manner. *E. coli* expression system is easy to use and the least expensive. A major drawback for bacterial host, however, is that it does not provide optimal milieu for the folding and assembly of human infecting viral antigens and enveloped virus antigen. Capitalizing on a novel function of RNAs as molecular chaperone (Chaperna), here we provide a robust protein folding vehicle that could be implemented for virus-like particle (VLP) or nanoparticle (NP) assemblies in bacterial host. Thus, a viral target surface antigen is fused with and RNA-interaction domain (RID), and expressed in *E. coli* as soluble form. The removal of RID by site-specific protease prompted the assemblage of monomers into regular multimeric complex, as confirmed by EM and DLS. We confirm that RNA is crucial for the folding of monomers and subsequent assembly into VLPs. The mutations that affects the RNA binding to RBD greatly increases the soluble aggregation into amorphous structures, reducing the overall yield of nanoparticles of defined size, confirming the importance of RNA interaction for the assembly into highly ordered complex. The results suggest that RNA functions as a ‘pace-maker’ for the assembly by controlling the overall kinetic network of antigen folding pathway into immunologically relevant conformation. The Chaperna platform holds promise to develop and deliver VLP and NP vaccines as a high-priority vaccine strategy against emerging viruses.

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Abstract of poster presentation / 発表要旨 【P-03】

Modification of HA glycosylation for better immunogenicity of avian influenza H7N9 vaccine

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Abstract

Recent cases of human infection with an avian influenza A(H7N9) virus in China have been a concern of public health. However, current vaccine candidates appeared to be less efficacious. To address a threat of the virus, we investigated molecular signatures of N-linked glycosylation (NLG) in the HA of H7 subtype viruses and their applicability for vaccine development. Based on the potential glycosites identified, we generated NLG mutant viruses using the HA of H7N9 A/Anhui/01/2013 on the 2009 pandemic H1N1 backbone. Unlike a single NLG-removed mutant from the HA residue 249 (rH7-249), single NLG-added (rH7+141, rH7+167) and double NLG-added (rH7+141+167) viruses produced similar or increased sizes of plaques in MDCK cells and exhibited better growth rates in MDCK, A549, and Vero cells, compared with a parental rH7 virus. Of the NLG mutants, rH7+141+167, which showed the highest replication property in the cells, appeared to induce the most broad-spectrum antibodies in the assays using guinea pig antisera. In an inactivated vaccine formulation, the double NLG-added vaccine protected mice from challenge by resulting in better immune responses than a NIBSC-made H7N9 vaccine. These suggest the strong potential of NLG modification for the development of a high yield, broad-spectrum vaccine against avian influenza viruses.

Keywords

avian influenza virus, hemagglutinin, immunogenicity, N-linked glycosylation

Acknowledgments

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Abstract of poster presentation / 発表要旨 【P-04】

Staphylococcal particulate cell wall releasing neutrophil-calprotectin has adjuvant activity as like aluminum salt

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Abstract

Nutritional immunity is a host defense response sequestrating host trace metals using neutrophil-derived calprotectin (S100A8/A9) to modulate bacterial growth and pathogenesis. But, the triggering signal(s) inducing release of calprotectin from neutrophils is not determined yet. Here, we found that both staphylococcal insoluble particulate cell wall (PCW, 1 μm particle size) and aluminum salt functioned as triggers for releasing calprotectin, but not by silica particle or staphylococcal soluble cell wall (SCW) that was prepared from PCW by treatment of peptidoglycan hydrolyzing enzymes. Upon injection of PCW or alum into murine peritoneal cavity, maximal large numbers of antigen presenting cells and high levels of neutrophil chemotaxin KC and monocyte chemotactic protein (MCP1) are detected after 12 h injection. Further, PCW- and alum-pre-injected mice, but not by silica- or SCW-injection, were highly protected from USA300 methicillin-resistant *Staphylococcus aureus* (MRSA) infection. As expected, PCW- or alum-injected peritoneal lavage fluids showed antibacterial activity in the presence of paraquat, but this activity was abolished by addition of manganese ion. Upon I.P. injection of PCW, both neutrophils and monocytes were recruited on the mediastinal lymph node after 6 h, while alum recruited monocytes only. Injection of ovalbumin/alum or ovalbumin/PCW mixture induced higher numbers of germinal centers in the nymph node and produced robust serum ovalbumin specific IgGs. Taken together, these results demonstrate that both staphylococcal PCW use calprotectin for induction of nutrition immunity and showed adjuvant activity as like aluminum salts.

Keywords
particulate cell wall, alum, calprotectin, adjuvant, nutrition immunity, Staphylococcus aureus

Abstract of poster presentation / 発表要旨　【P-05】

Neuropeptides control the pathology of severe influenza virus infection

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Severe influenza virus infection is characterized by extremely high cytokine production and virus replication with high mortality rate. Catechlamines (CA) have been shown to modulate pulmonary inflammation and immune responses through both, neural and non-neural phagocyte-dependent mechanisms. Besides CA, sympathetic nerves also release neuropeptide Y (NPY) upon stimulation. However, it remains unknown if any non-neural sources of NPY may also play a role in the response to severe influenza virus infection. Here we show that de novo synthesis of NPY was increased in phagocytes including alveolar and interstitial macrophages, and monocytes following influenza virus infection in lungs. Genetic deletion of Npy or its receptor specifically in myeloid cells greatly improved the pathology of severe influenza virus infection. NPY signalling on phagocytes impaired anti-viral response and promoted pro-inflammatory cytokine production, and thereby enhancing the pathology of influenza virus infection. Thus, direct regulation of NPY-its receptor axis in phagocytes could act as a fine-tuner of an innate immune response to virus infection, which could be a novel therapeutic target for severe influenza virus infection.

Abstract of poster presentation / 発表要旨　【P-06】

Assessment of K3-SPG, nano-particulate CpG DNA, as an immunotherapeutic agent in non-human primate models *in vivo*

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【Introduction】Priming, boosting and restoring memory cytotoxic T lymphocytes (CTLs) by vaccination or by immunotherapy in vivo have been actively investigated. While immunostimulatory CpG DNA attract attention to elicit strong antigen-specific CTL responses as a vaccine adjuvant, very few have been shown to act as an anti-cancer mono-therapeutic agent particularly in non-human primates (NHP). In this study, we used cynomolgus macaques to estimate the potential of K3-SPG, the complex of CpG oligodeoxyribonucleotides (K3) and schizophyllan (SPG) for TLR9 synthetic ligand, as an immunotherapeutic agent.

【Methods】We used 3 different nucleic acids-based adjuvants in this study; K3, K3-SPG and poly (I:C) for TLR3, Retinoic acid-Induced Gene-I (RIG-I), Melanoma Differentiation Associated gene 5 (MDA5) ligands. After administration of either K3, K3-SPG or poly (I:C) in cynomolgus monkeys, a complete blood count was determined. And we also measured the cytokines/chemokines production in serum and analysed the frequencies and activation stats of neutrophils and T cells in peripheral blood mononuclear cells by flow cytometry.

【Results】We show that a single injection subcutaneously or intravenously of K3-SPG significantly increased the frequencies of activated memory $CD8^+$ T cells in a NHP model. Vaccine-induced, antigen-specific memory $CD8^+$ T cells were boosted by following administration of K3-SPG alone, but not other agonists K3 or poly (I:C). Although K3-SPG induced systemic inflammation, the levels of neutrophil influx in the blood and proinflammatory cytokines in serum were lower than those induced by poly (I:C) injection.

【Discussion】K3-SPG may represent a potent mono-immunotherapeutic agent to boost antigen-specific memory CTL responses even in the absence of specific antigen without robust systemic inflammation.

Abstract of poster presentation / 発表要旨　【P-07】

An approach to establish an *in vitro* assay system for the safety control of influenza vaccines and adjuvants

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BACKGROUND

The biological responses in the vaccinated animals are tested as indicators for the safety of vaccines. With the recent development of cDNA microarray technology, it has become possible to identify genes related to the safety of vaccines. We have identified 17 marker genes whose expression was enhanced in vaccinated animals' lungs upon treatment of whole virion influenza vaccine, and found that these markers could also be applied to the evaluation of influenza vaccines containing adjuvants. For the next step, the establishment of an i*n vitro* assay system would be desired as an alternative to animal tests for the safety assessment of HA vaccines and adjuvants.

METHODS

Adjuvants were serially diluted, mixed with influenza HA vaccines, and added to cell lines derived from lung tissue of rat, mouse and human origin. Reference influenza vaccine (inactivated whole virion vaccine) was used as a positive control. After 24 hours, the cells were lysed, and the expression levels of marker genes were measured using branched DNA-based method. Cytokines/chemokines released from the cells were also analyzed.

RESULTS

Reference influenza vaccine induced the upregulation of marker genes in a dose-dependent manner. The responsiveness to reference influenza vaccine varied in each cell line. HA vaccine containing Alum adjuvant could induced the moderate upregulation of marker genes, whereas HA vaccines containing poly(I:C) or K3 adjuvants showed marginal or no significant effects on the expression profile of marker genes. It was consistent with the result that cell lines which were used in this study expressed Toll-like receptor (TLR) 3, but not TLR9-specific mRNA. The release of chemokines was sometimes shown even when the upregulation of marker genes was not obvious.

CONCLUSION

The expression analysis of marker genes in the *in vitro* assay system will be expected to apply to the safety evaluation of influenza vaccines and adjuvants. However, our assay system may not be optimal for adjuvants which were designed to target TLRs, especially TLR9. Chemokines could be other markers for the safety evaluation of influenza vaccines and adjuvants.

Abstract of poster presentation / 発表要旨　【P-08】

Enhancement of avidity and ADCC activities of virus-specific antibodies by TLR agonists improved vaccine efficacy of influenza split vaccines

TLR アゴニストによる抗体の avidity の増強または ADCC 抗体の産生誘導はインフルエンザスプリットワクチンの防御効果を増強する

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Influenza vaccines induce protective immunity via induction of antibody production against subsequent infections, and a variety of different vaccine types are currently available. We have previously reported that split influenza vaccines induce higher levels of virus-specific antibodies compared to whole-virion vaccines upon responses to a booster immunization. To identify the mechanism of these antibody inductions, the effects of several TLR agonists were determined. Mice were injected with split vaccines with or without several TLR agonists twice with a 4-week interval and the sera were obtained 2 weeks after the last immunization. Vaccine-specific IgG, hemagglutination-inhibition antibody (HI) titers, microneutralization antibody (MN) titers, avidity of vaccine-specific IgG, and ADCC activities in the sera were examined. Split vaccines with TLR7 and TLR9 agonists induced significantly higher avidity of vaccine specific IgG than those with TLR3 agonist or without adjuvant. On the other hand, vaccines with TLR3 and TLR9 agonists induced ADCC activities in sera but those with TLR7 agonist did not. HI and MN titers were comparable among vaccines with each agonist. In addition, split vaccines with all agonists significantly decreased virus titer in the lung after subsequent infection, as compared with those in the absence of TLR agonist. Further studies are underway to clarify the mechanism of antibody induction by vaccines with TLR agonists and to identify the profile of antibodies, which correlate to the virus elimination.

Abstract of poster presentation / 発表要旨 【P-09】

Serum bactericidal assay for the evaluation of typhoid vaccines using a semi-automated colony counting method

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ABSTRACT

Typhoid fever, mainly caused by *Salmonella enterica* serovar Typhi (S. Typhi), is a life-threatening disease, mostly in developing countries. Enzyme-linked immunosorbent assay (ELISA) is widely used to quantify antibodies against *S*. Typhi in serum but does not provide information about functional antibody titers. Although the serum bactericidal assay (SBA) using an agar plate is often used to measure functional antibody titers against various bacterial pathogens in clinical specimens, it has rarely been used for typhoid vaccines because it is time-consuming and labor-intensive. In the present study, we established an improved SBA against *S*. Typhi using a semi-automated colony-counting system with a square agar plate harboring 24 samples. The semi-automated SBA efficiently measured bactericidal titers of sera from individuals immunized with *S*. Typhi Vi polysaccharide vaccines. The assay specifically responded to *S*. Typhi Ty2 but not to other irrelevant enteric bacteria including *Vibrio cholerae* and *Shigella flexneri*. Baby rabbit complement was more appropriate source for the SBA against *S*. Typhi than complements from adult rabbit, guinea pig, and human. We also examined the correlation between SBA and ELISA for measuring antibody responses against *S*. Typhi using pre- and post-vaccination sera from 18 human volunteers. The SBA titer showed a good correlation with anti-Vi IgG quantity in the serum as determined by Spearman correlation coefficient of 0.737 ($P < 0.001$). Taken together, the semi-automated SBA might be efficient, accurate, sensitive, and specific enough to measure functional antibody titers against *S*. Typhi in sera from human subjects immunized with typhoid vaccines.

Abstract of poster presentation / 発表要旨　【P-10】

Relationship between the inducibility of the IFN-β production in Sendai virus Cantell strain and the antagonistic action toward the IFN-α/β signal transduction by the C protein

センダイウイルス カンテル株における IFN シグナル伝達阻害作用と IFN-β 産生誘導能の関係性の解明

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Viral nucleic acids, which activate the innate immunity responses of the host, can be used as a vaccine adjuvant to increase the immunogenicity. Sendai virus (SeV) is known as a virus causing the acute respiratory infection in mice. Cantell strain of SeV produces a large amount of defective interfering (DI) RNA, which is easily recognized by host, inducing the production of IFN-β to activate the innate immunity (Yoshida *et. al.*, ***J. Virol.***, in press). There is no report that SeV infects human, although it exhibits a high cell fusion activity. In addition, since SeV has little carcinogenic risk to human due to the inability of the genome to invade the host nucleus during viral life cycle, SeV is frequently used as a vector to express foreign proteins in the cell by using genetic recombination. Therefore, Cantell strain has a potential as an excellent vaccine adjuvant. In SeV, C protein is responsible for the suppression of IFN-α/β signaling to the nucleus, since it inhibits the tyrosine phosphorylation of Signal Transducer and Activator of Transcription 1 (STAT1) and STAT2. The C protein also suppresses the IFN-γ signaling, by inhibiting the activation of the STAT1 homodimer. On the other hand, production of IFN-β is known to be suppressed by negative feedback (Jin and Cui, ***Autophagy***, 2017; Jin *et. al.*, ***Mol. Cell***, 2017). Therefore, inducible IFN-β production by DI RNA may be positively regulated by the inhibitory action of the C protein to the IFN-α/β signal transduction.

C-terminal domain of the C protein, named Y3, binds to the N-terminal domain of STAT1 (STAT1ND). Our X-ray crystallographic study demonstrated that the two molecules of Y3 symmetrically bind to each niche created in the STAT1ND dimer. By comparing with the structure of full-length STAT1, we can clarify the structural mechanism underlying the STAT1 inactivation by the C protein (Oda *et. al.*, ***J. Virol.***, 2015). On the other hand, N-terminal domains of the STAT family proteins are thought to be unable to form heterodimeric structures. However, we revealed that STAT1ND can associate with STAT2ND by SAXS analysis using a recombinant protein, in which STAT1ND fuses with STAT2ND via a flexible linker peptide. Furthermore, we demonstrated that one molecular of C protein associates with STAT1ND:STAT2ND heterodimer. These results suggest that the binding of the C protein to the STAT1:STAT2 heterodimer induces the change of the conformation to the antiparallel form, leading to the dephosphorylation (Oda *et. al.*, ***J. Biol. Chem.***, 2017).

In the future, we would like to create recombinants of the SeV Cantell strain as a desirable vaccine adjuvant by introducing the mutation into the C protein gene to alter the inhibition activity against IFN-α/β signaling, which is expected to have a high inducibility of IFN-β production.

Abstract of poster presentation / 発表要旨 【P-11】

Th17 promotes the induction of antigen-specific gut-mucosal cytotoxic T lymphocytes following intramuscular vaccination of an adenovirus vector / アデノウイルスベクターワクチンによる腸管粘膜面での抗原特異的細胞傷害性 T 細胞の誘導は Th17 によって促進される

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Most pathogens access the body through mucosal membranes; therefore it is a high priority in global health to develop vaccines capable of establishing protective immune responses at both systemic and mucosal sites. However, induction of mucosal immunity by systemic administration of vaccines has proven difficult due to the unique immunological features of the mucosal immune system. Reports showed that intramuscular (*i.m.*) vaccination of an adenovirus vector (Adv) induces functional and sustainable antigen (Ag)-specific cytotoxic T lymphocytes (CTLs) in gut-mucosal compartments, as well as systemic compartments, in mice and rhesus macaques. Therefore, an Adv has potential as a next-generation mucosal vaccine. Adv-derived nucleic acids, adenoviral genomic DNA and noncoding RNA, trigger innate immune responses through several pathways, resulting in robust production of type I interferons (IFNs). We previously revealed, using *Ifnar2*$^{-/-}$ mice, that type I IFN signaling is required for induction of gut-mucosal, but not systemic, CTLs following vaccination; however, the molecular mechanism elicited by type I IFN signaling remains unknown. In the present study, we revealed that Ag-specific T helper 17 (Th17) cells were reduced in gut mucosa of *Ifnar2*$^{-/-}$ mice following *i.m.* Adv vaccination. Here, we showed that transfer of Th17 cells into *Ifnar2*$^{-/-}$ mice prior to Adv vaccination rescued the induction of Ag-specific gut-mucosal CTLs. Interestingly, the transferred Th17 cells enhanced the induction of Ag-specific CTLs in gut mucosa, but not in systemic sites in wild-type mice. These data suggested that Th17 cells translate systemic type I IFN signaling into gut-mucosal CTL response following *i.m.* Adv vaccination. We believe that our results will promote the development of advanced mucosal vaccines based on the novel concept of Th17 induction.

Abstract of poster presentation / 発表要旨　【P-12】

Organogenesis of inducible bronchus-associated lymphoid tissue plays an essential role in Ag85B-rhPIV2-based anti-tuberculosis respiratory vaccine in mice

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Abstract

We previously reported that Ag85B-expressing human parainfluenza type 2 virus (Ag85B-rhPIV2) was useful as a nasal vaccine against tuberculosis; however, its mechanism underlying immune induction remains to be investigated. In this study, we found that the organogenesis of inducible bronchus-associated lymphoid tissue (iBALT) played an essential role in the induction of antigen-specific T and B immune responses in the lung. Organogenesis of iBALT was induced by intranasal administration of Ag85B-rhPIV2 or null-hPIV2 vector without excessive inflammation. When iBALT genesis was disrupted by depletion of $CD11b^+$ cells or impairing lymphotoxin-signalling, Ag85B-specific immune responses (i.e., IFNγ-producing T cells and IgA antibody) were diminished in the lung. These results suggest that iBALT plays a key role in the induction of antigen-specific immune responses in Ag85B-rhPIV2-based anti-tuberculosis vaccine as a safe and efficient nasal vaccine.

Abstract of poster presentation / 発表要旨　【P-13】

Prime-boost Vaccination with CpG ODN and curdlan strongly induces both systemic and mucosal immunity / CpG ODN とカードランを用いたプライム - ブーストワクチン法は全身免疫と粘膜免疫の両方を強く誘導する

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The injectable vaccines used today induce pathogen-specific IgG and Th1 responses to halt the progression of infectious diseases. However, most pathogens enter the body through the mucosa, and the induction of pathogen-specific mucosal immunity involving secretary IgA (SIgA) and Th17 responses is essential for inhibiting their entry. Unfortunately, injectable vaccines are poor inducers of antigen (Ag)-specific mucosal immunity, because it is considered to be induced by unique mucosal dendritic cell (DC) subsets. Our previous studies showed that $CD103^+CD11b^+$ DCs in small intestinal lamina propria (siLPDCs) induce intestinal mucosal immunity by Toll-like receptor ligand stimulation via flagellin or CpG oligodeoxynucleotides (CpG-ODN). Unlike splenic DCs (SPDCs), they specifically express retinoic acid-converting enzyme retinal dehydrogenase isoform 2 (RALDH2), and can induce Ag-specific SIgA and Th17 responses in addition to IgG and Th1 responses. Here, we show that SPDCs co-stimulated by CpG-ODN and curdlan induce similar immune responses to those induced by activated siLPDCs. Intramuscular Ag injection with CpG-ODN and curdlan induces Ag-specific systemic responses (e.g., IgG and Th1 production). The priming immunization also induces Ag-specific SIgA in stool, thereby protecting against cholera toxin-induced diarrhoea. Although the Ag-specific SIgA induction is transient after priming, oral Ag administration induces high levels of Ag-specific IgA in stools that exceeded 3 months. Moreover, Ag-specific Th1 and Th17 responses are induced in intestine after boosting. The boosting effect is also induced in the lungs and vaginas by intranasal and intravaginal Ag administration, respectively. This prime-boost method would provide an innovative injectable hybrid mucosal vaccine capable of effectively preventing pathogen invasion and disease progression in the targeted mucosal tissues.

Abstract of poster presentation / 発表要旨　【P-14】

T cell response analysis against human herpesvirus 6B glycoprotein complex vaccination

ヒトヘルペスウイルス 6B の糖タンパク質複合体に対する T 細胞免疫応答

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Human herpesvirus 6B (HHV-6B) belongs to betaherpesvirus subfamily which is a causative agent for exanthem subitum and is sometimes associated with severe encephalopathy in immunosuppressed patients. A glycoprotein (g) H/gL/gQ1/gQ2 complex that is unique to HHV-6, is a viral ligand for its cellular receptor. In this study, we examined this HHV-6B glycoprotein complex vaccination induced T cell responses in mice by using a peptide library consisted of 162 peptides covering the whole gH, gL, gQ1, and gQ2 sequences.

BALB/c mice and C57BL/6J mice were immunized intradermally with the gH/gL/gQ1/gQ2 complex and various vaccine adjuvants, and then splenocytes were stimulated *in vitro* with 162 peptides and gamma interferon (IFN-γ) production was determined. We found that adjuvant X induced strong T cell responses for the gH/gL/gQ1/gQ2 complex vaccination in mice. We further investigated the T cell responses induced with gH/gL/gQ1/gQ2 complex plus adjuvant X by depleting $CD4^+$ or $CD8^+$ T cells with MACS system. Depletion experiment revealed that the gH/gL/gQ1/gQ2 complex vaccination with adjuvant x dominantly induced $CD4^+$ T cell response in both BALB/c and C57BL/6J mice. Only weak $CD8^+$ T cell responses were detected in C57BL/6J mice. These result suggested that the gH/gL/gQ1/gQ2 complex vaccination can induce strong CD4 T cell responses in mice, and speculate to have a similar result in human.

Abstract of poster presentation / 発表要旨　【P-15】

Cooperative Adjuvant Effect of Combination of 2-Hydroxypropyl-β-Cyclodextrin and CpG-ODN

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Adjuvants are used in vaccine formulations to induce potent immune responses that cannot be obtained with antigen alone. However, sufficient immune responses may not be shown with antigen and an adjuvant, thus the combination of multiple adjuvants has also been developed. Recently, Onishi *et al*. reported that 2-hydroxypropyl-β-cyclodextrin (HP-β-CyD) has Th2 adjuvanticity and produces low IgE, which is involved in allergic reaction[1], suggesting the possibility of HP-β-CyD as a novel adjuvant with few side effects. However, there is no report on the combination of HP-β-CyD and the other adjuvants. Meanwhile, Alum or CpG-ODN is known as one of promising adjuvants for Th2 response or Th1 response, respectively. Therefore, in the present study, we investigated the cooperative adjuvant effects of HP-β-CyD in combination with Alum (Th2 response) and CpG-ODN (Th1 response).

C57BL/6 mice were administered ovalbumin (OVA) solutions containing HP-β-CyD and Alum or CpG-ODN into the base of the tail on day 0 and 14. On day 21, the blood was collected, and anti-OVA IgG1 (Th2 indicator) and IgG2c (Th1 indicator) in serum were determined by ELISA. As a result, production of IgG1 after administration of HP-β-CyD with Alum was almost comparable to that of Alum alone, and that after administration of HP-β-CyD with CpG-ODN was equivalent to that of HP-β-CyD alone. Meanwhile, the production of IgG2c after administration of HP-β-CyD with CpG-ODN was significantly increased, compared to that of HP-β-CyD alone and CpG-ODN alone. In addition, we examined whether HP-β-CyD and CpG-ODN activate B cells and dendritic cells in draining lymph node after co-administration in C57BL/6 mice. Consequently, HP-β-CyD and CpG-ODN cooperatively upregulated the expression of co-stimulatory molecules on $CD19^+$ cells (B cells), whereas such effect was not shown on $CD11c^+$ cells (dendritic cells). Taken together, these results suggest that the combination of HP-β-CyD and CpG-ODN induces both Th1 and Th2 responses and particularly enhances Th1 response, due to the activation of B cells.

Reference:
(1) M. Onishi *et al.*, ***J. Immunol.***, 194, 2673-2682 (2015).

Abstract of poster presentation / 発表要旨　【P-16】

Adjuvant X can induce both cellular and humoral immunity even at very low antigen dose
低抗原量でも細胞性および液性免疫を誘導するアジュバントＸ

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Development of T cell response inducing vaccine is important, especially for a new influenza virus pandemic such as H5N1 and H7N9, because most of people do not have neutralizing antibody against these new viruses, and it has been shown that T cells induced by seasonal influenza virus infection can be responding and protective against these new virus infections. However, induction of T cell responses by current protein vaccines are very limited even using a relatively strong adjuvant because larger amount of antigen is required to induce detectable T cell responses than antibody responses. We demonstrated that adjuvant X efficiently induced both cellular and humoral immune responses upon very low dose of antigen immunization(s), compared with other strong adjuvant such as cdiGMP and P-type ODNs. These results indicated that adjuvant X is a promising future vaccine adjuvant candidate to induce T cell responses against emerging infectious diseases with clinically applicable antigen dose.

Abstract of poster presentation / 発表要旨　【P-17】

Induction of IL-12p70 by a novel oligonucleotide in human PBMC
新規免疫賦活化核酸によるヒト PBMC における IL-12 の誘導メカニズムの解明

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Vaccines need to initiate innate immune responses to induce protective adaptive immune responses. Adjuvants are substances added to vaccines to induce innate immune responses, activating antigen presenting cells (APCs), consequently enhancing immunogenicity of highly purified vaccine antigens that have usually insufficient immunostimulatory capabilities. CpG oligonucleotides (ODNs) have been used as a vaccine adjuvant, and have been known to induce cytokines including IFN-α, IL-6, and IL-12p70 production, promoting Th1 type immune responses. IFN-α and IL-6 induction by CpG ODNs was well studied, however, there have been few reports about CpG ODNs inducing IL-12p70 production in human PBMCs.

We screened more than 500 kinds of CpG related oligonucleotides, among these, we found a ODN (ODN-I) which can uniquely induce strong IL-12p70 production in human PBMCs. Although previous report showed that human dendritic cells are the major source of IL-12p70 production, our current result suggested that DCs (CD1c+ mDCs, CD141+ mDCs and pDC) were not a major source of IL12p70 after ODN-I stimulation in human PBMCs.

Abstract of poster presentation / 発表要旨　【P-18】

Vaccine adjuvant effects of dendritic cell-targeting peptides

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Subunit vaccines are expected with improved safety compared with other vaccine types. However, subunit vaccines must be delivered to dendritic cells (DC) more efficiently and administered with an adjuvant, because the vaccine antigen alone does not induce sufficient protective immunity. Our group is developing novel targeting peptide as an antigen delivery carrier by using a phage display system, which is an *in vitro* screening technique that uses a library of bacteriophages displaying various peptides. In the present study, we tried to identify peptides that bind dendritic cells (DC) as antigen delivery carrier for vaccine development by means of phage display system. A bacteriophage library was mixed with bone marrow-derived DC and the bacteriophage clones that bound the DC were recovered. The binding capacity of the recovered bacteriophage clones was determined by flow cytometry: many of the peptides that bound the DC have a common sequence motif. We examined the vaccine adjuvant effects of the DC-binding peptides in mice by using recombinant fusion to conjugate the identified peptides with an antigen (antigen–peptide). The antigen-specific antibody response in mice administered antigen–peptide was significantly higher than in mice administered the antigen alone and comparable to in mice administered the antigen + adjuvant. Next we evaluated the usefulness of the peptide (FL4 peptide) as an antigen delivery carrier in mice to optimize cancer vaccine. We used ovalbumin (OVA)-derived peptide ($OVA_{257–264}$; SL8) as antigen, and CpG oligodeoxynucleotide (ODN; a TLR9 ligand) as adjuvant. SL8 or the fusion peptide SL8-FL4 without CpG ODN could not induce SL8-specific cytotoxic T lymphocytes (CTL). The SL8-specific CTL response in mice treated with SL8-FL4 plus CpG ODN was significantly higher than that in mice treated with SL8 plus CpG ODN. In addition, SL8-FL4 plus CpG ODN induced a strong antitumor effect against OVA-expressing EG7 tumor cells. Together, these results suggest that the identified DC-targeting peptides are potentially useful antigen-delivery carrier for vaccines.

Abstract of poster presentation / 発表要旨　【P-19】

Efficient induction of CD8$^+$ cytotoxic T lymphocytes response by mutant dendritic cell-targeting peptide

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It is believed that antigen specific CD8$^+$ cytotoxic T lymphocytes (CTLs) play an important role in preventing infectious diseases and cancer. However, most reported vaccines cannot induce sufficient CTLs for rejection of infected or malignant cells, because an efficient way to deliver the antigen to dendritic cells (DCs) is lacking. In a previous study, we identified many peptides that bind to DCs. Furthermore, we showed that one of these peptides (FL4 peptide) when fused with antigen, efficiently promotes not only antigen specific antibodies but also CTLs responses. This finding suggests that FL4 peptide might be an efficient way to deliver antigen to DCs to induce antigen-specific CTLs. In this study, to develop more effective vaccine, we assessed the capacity of FL4 mutant peptides to induce CTL responses. We used ovalbumin (OVA)-derived peptide ($OVA_{257–264}$; SL8) as antigen, and CpG oligodeoxynucleotide as adjuvant. The SL8-specific CTL response in mice treated with a fusion peptide SL8-M6 (one of the FL4 mutant peptides) plus CpG ODN was significantly higher than that in mice treated with SL8-FL4 or the other mutant fusion peptides plus CpG ODN. In addition, SL8-M6 plus CpG ODN induced a strong antitumor effect against OVA-expressing EG7 tumor cells. These results suggest that M6 peptide has potential to induce CTLs effectively. We are currently assessing that mechanism of CTL-induction by SL8-M6.

Abstract of poster presentation / 発表要旨　【P-20】

Activation of $CD4^+$ T cell response by using dendritic cell-targeting peptide

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It is commonly believed that $CD8^+$ T cell responses play a major role in antiviral immunity. Although this remains true for many viruses, several studies recently have shown that $CD4^+$ T cells are also important in antiviral response. Therefore, the development of vaccine that can induce $CD4^+$ T cell responses efficiently is necessary. In this study, we tried to develop novel vaccine which could enhance $CD4^+$ T cell response by delivering antigen to dendritic cells (DCs). To identify DC-targeting peptides, we used a phage display system to screen a library of bacteriophages that could bind DCs by displayed peptides and identified the DC-targeting peptide. Next, we evaluated the usefulness of this DC-targeting peptide as an antigen delivery vehicle in mice to induce antigen specific $CD4^+$ T cell response. We used ovalbumin (OVA)-derived peptides (MHC class I epitope $OVA_{257–264}$; SL8 and MHC class II epitope $OVA_{323-339}$; OTII) as antigens, and CpG oligodeoxynucleotide (ODN; a TLR9 ligand) as an adjuvant. OTII-specific $CD4^+$ T cell response in mice treated with the OTII fused with the DC-targeting peptide plus CpG ODN was significantly higher than that in mice treated with OTII or control fusion peptides plus CpG ODN. In contrast, the SL8 fused with the DC-targeting peptide plus CpG ODN could not induce SL8-specific $CD8^+$ T cell response. These results suggest that our identified DC-targeting peptide might be useful as an antigen-delivery vehicle to activate antigen specific $CD4^+$ T cell response.

Abstract of poster presentation / 発表要旨　【P-21】

Mechanism of synthetic hemozoin adjuvanticity

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GLP-manufactured synthetic hemozoin (sHZ) is a beta-hematin polymer produced from hemin chloride that yields around 1 μm crystals with optimal antigen-specific adjuvant effects. This simple-to-produce adjuvant candidate has the added advantage of stability and cost-effectiveness besides its known adjuvanticity and safety, making it rational to further investigate its potential as vaccine adjuvant. Although sHZ behaves like a type-2 adjuvant, the adjuvant effects of sHZ are dependent on the model antigens and the host species. Several studies have suggested the benefits of sHZ as an adjuvant, such as reduction of IgE production in dog allergy model immunized with allergen, and non-pyrogenic sterile protection against influenza when immunized with HA split vaccine in ferrets. The potency of sHZ adjuvanticity and its safety as adjuvant have been assessed, but the mechanism of action has not been investigated. Here we demonstrate the mechanism of adjuvanticity and enhanced class-switching mediated by sHZ.

Abstract of poster presentation / 発表要旨 【P-22】

ZBP1 governs neutrophil-mediated inflammation in influenza virus infection via IL-1α

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Keywords: influenza, cell death, NLRP3 inflammasome, IL-1α/β, neutrophils,

In influenza A virus (IAV) infection, IL-1 plays an important role in both protective and pathological immunity, however, the precise immunological mechanism by which the production of IL-1α and IL-1β is regulated during lethal and/or non-lethal IAV is unknown. Recent evidence suggest that Z-DNA binding protein-1 (ZBP1) is involved in IAV-induced cell death and IL-1β induction, however, the involvement of the NLRP3 inflammasome is unclear, and ultimately it remains controversial if ZBP1 contributes toward protective, or pathological immunity.

Here, we show that ZBP1 is essential not only for IAV-induced cell death and IL-1β induction, but also for IL-1α induction, which did not require the NLRP3 inflammasome pathway. Indeed, mice lacking NLRP3, ASC or Caspase-1/11 had no phenotype, while those lacking either IL-1 receptor (IL-1R) or ZBP1 displayed higher viral load and mortality than wild type mice. Conversely, when mice were infected intratracheally, to mimic highly pathogenic infection, ZBP1 deficiency protected mice from acute pulmonary inflammation. These reciprocal roles of ZBP1 mirrors its essential role in recruitment and infiltration of neutrophils to the lung, and in resultant inflammation and neutrophil extracellular trap (NET) formation.

Collectively, these results indicate that ZBP1 is essential not only for IAV-induced cell death, but also IL-1α production in vitro and in vivo in the absence of NLRP3 inflammasome machinery. As a result, ZBP1 controls IL-1-dependent, neutrophil-mediated pulmonary inflammation, leading to reciprocal outcomes, protective immunity or immunopathology, depending on the type, kinetics, and target host tissue of IAV infection.

Abstract of poster presentation / 発表要旨 【P-23】

The role of Interleukin-1α and DNA in the particles-associated inflammation

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Abstract summary

Recently, the number of patients with allergic inflammation, including allergic asthma or rhinitis have been increased. Although the reason is unclear, many reports suggest that environmental factors are causing allergic inflammations, one of well-known factors is particle pollution, such as particulate matter 2.5 (PM2.5). PM2.5 is a micro-sized particle and these kind of fine particles are known to enter deep in the lung and to induce pulmonary inflammation. In addition, recent studies have revealed that these fine particle function as an adjuvant to induce allergic immune responses.

Adjuvants are the reagents that enhance the immune response to coadministered antigen(s). Once particle adjuavnts are inhaled, they are deposited in the lung, and then induce in allergic lung inflammation, which is characterized by increase serum immunoglobulin E (IgE) levels against allergen. In general, IgE is a well-known mediator and an important marker for allergic diseases.

Aluminium salts (referred to as Alum) are the most commonly-used-particle adjuvants, and are known to preferentially increase the IgE level. However, underlying mechanisms of Alum' adjuvanticity and induction of IgE responses are still unclear. One of proposed mechanisms of action of Alum is reported that damage-associated molecular patterns (DAMPs), including DNA, are released under the cell death and activate the innate immune cells.

Furthermore, we previously showed that Interleukin-1 alpha (IL-1α) works as a DAMP and is released under the cell death after Alum stimulation, in addition to host DNA release. We also observed that IL-1α were released in the lung after administration of Alum into the lungs, and release IL-1α is involved in IgE production in vivo.

In this study, we investigated the role of IL-1α and DNA as DAMPs, and we hypothesized that IL-1α bind to DNA and is released as a DNA- IL-1α complex under cell death for effective activation of immune cells.

References

1) Kuroda et al. Immunity 45.6 (2016): 1299-1310
2) Marichal et al. Nature medicine 17.8 (2011): 996-1002.

Abstract of poster presentation / 発表要旨　【P-24】

Immunological mechanism of synergistic anti-cancer activities by activation of TLR9 and STING

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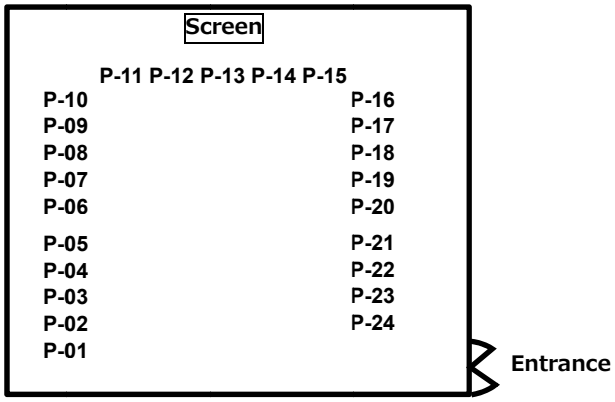
TLR9 and Stimulator of IFN Gene (STING) agonists are both potential anti-tumor agents and vaccine adjuvants that have been used in clinical trials. However, several concerns are raised as currently available TLR9 agonist, CpG oligodeoxynucleotide (ODN) (K-type), is a weak inducer of IFNs and STING agonists rather induce type-2 immune responses, limiting their potential therapeutic applications. Our previous report demonstrated that TLR9 and STING agonists synergize for the induction of innate and adaptive type-II IFN via the mechanisms involving type-I IFNs and IL-12. In addition, together TLR9 and STING agonists become a potent type-1 adjuvant and an anti-tumor agent that can elicit robust CTL- and NK cell-dependent anti-tumor immunity in mouse tumor models of thymoma and melanoma, respectively. Here, we further show and characterize the anti-tumor effect of combination of K3 CpG (TLR9 agonist) and cyclic dinucleotides (STING agonists) in the pancreatic cancer model using Pan02 cells. By using the Pan02 peritoneal dissemination model, we found that local but not systemic combination treatment exert a potent anti-tumor effect. Moreover, combination treatment is superior to K3 CpG- and STING agonist-only groups in the induction of long term survivors that recovered from cancer. Furthermore, these long term survivors are also resistant to a secondary challenge with Pan02 cells, suggesting the development of the tumor-specific memory responses by the combination treatment. Finally, we demonstrated that co-ordinate action of IL-12 and type-I IFNs is required for the anti-tumor effect of the combination in the Pan02 peritoneal dissemination model, which is consistent with or previous findings indicating that IL-12 and type-I IFNs produced by APCs are necessary for mediating the synergistic effect of combination on innate and adaptive IFNg induction. Together, these data reveals the potential of the combinatorial use of TLR9 and STING agonists in cancer immunotherapy for multiple cancer types.

ポスター発表＜Poster　Layout＞

【掲示時間】平成 30 年 1 月 23 日（火）10:00～14:00
※うち、ポスターセッション　13:15～14:00
【掲示場所】サイエンスホール



P-01	Man ki Song（International Vaccine Institute，韓国）
P-02	Baik Lin Seong （Yonsei University，韓国）
P-03	Man-Seong Park（ Korea University College of Medicine，韓国）
P-04	Bok Luel Lee（ Pusan National University，韓国）
P-05	今井　由美子(NIBIOHN)
P-06	升田　雄士（NIBIOHN、大阪大学、日本新薬(株)）
P-07	百瀬　暖佳（ 国立感染症研究所）
P-08	佐藤　佳代子（ 国立感染症研究所）
P-09	Jae Seung Yang（International Vaccine Institute，韓国）
P-10	小田　康祐（広島大学）
P-11	立花　雅史（大阪大学）
P-12	長竹　貴広（NIBIOHN）
P-13	武村　直紀（千葉大学大学院、東京大学）
P-14	奥谷　三慧（(財)阪大微生物病研究会、大阪大学 ）
P-15	林　智哉（NIBIOHN、大阪大学）
P-16	長谷田　泰成（大阪大学）
P-17	孟 潔（大阪大学）
P-18	三里一貴（ 大阪大学）
P-19	金井優紀（ 大阪大学）
P-20	白井星記（ 大阪大学）
P-21	Michelle Sue Jann LEE（Osaka University）
P-22	百田　匡寿（NIBIOHN，大阪大学）
P-23	日置 仰 （大阪大学, NIBIOHN）
P-24	BURCU TEMIZOZ（Osaka University）

Today's Program

○2nd ISV Asia Vaccine and Immunotherapeutic Symposium (10:00-12:10)

10:00-10:05	Opening remarks: Margaret Ann Liu / International Society for Vaccines
10:05-10:30 (25min)	Jerome Kim / International Vaccine Institute, Korea
10:30-10:55 (25min)	Arnaud Didierlaurent / Glaxo Smith Kline plc, Belgium
10:55-11:20 (25min)	Hiroshi Kiyono / The Institute of Medical Science, the University of Tokyo (IMSUT)
11:20-11:40 (20min)	Kouji Kobiyama / La Jolla Institute for Allergy and Immunology, USA
11:40-11:55 (15min)	Juine-Ruey (JR) Chen / Adimmune Corporation，Taiwan
11:55-12:10 (15min)	Guanghui Ma / Institute of Process Engineering, Chinese Academy of Sciences

○Poster Session / Coffee-break (13:15-14:00)

○11th Meeting of the Japanese Vaccine Adjuvant Research Consortium (14:00-17:30)

14:00-14:05	Opening remarks : Koichi Yamanishi / The research foundation for microbial disease of Osaka University)
14:05-14:30 (25min)	Sho Yamasaki / Osaka University
14:30-14:55 (25min)	Satoshi Uematsu / Chiba University・The University of Tokyo
14:55-15:20 (25min)	Etsushi Kuroda / National Institutes of Biomedical Innovation, Health and Nutrition（NIBIOHN）
15:35-16:00 (25min)	Katsufumi Nakayama / Tohoku University
16:00-16:15 (15min)	Eita Sasaki / National Institute of Infectious Disease (NIID)
16:15-16:30 (15min)	Shinsuke Inuki / Kyoto University
16:30-16:55 (25min)	Katsuyo Ohashi-Doi / Torii Pharmaceutical Co., Ltd.
16:55-17:20 (25min)	Hironori Nakagami / Osaka University
17:20-17:25	Closing remarks : Yoshihiro Yoneda / National Institutes of Biomedical Innovation, Health and Nutrition（NIBIOHN）

第 11 回次世代アジュバント研究会　プログラム・講演要旨集

Program and Abstracts (January 23, 2018, Senri Life Science Center, Osaka,Japan)

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