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COBRA: Computationally-Optimized Broadly Reactive Antigen Vaccine Against Novel H1N1 and Seasonal H1N1 Influenza Strains

One of the challenges for developing influenza A and B vaccines are the diversity of antigenically distinct isolates within each subtype. Previously, our group described a novel hemagglutinin (HA) for H5N1 influenza derived from a methodology termed computationally optimized broadly reactive antigen (COBRA). This COBRA HA, when used as an immunogen, elicited a broad antibody response against H5N1 isolates from different clades. We now report the development and characterization of a COBRA-based vaccine for all seasonal and pandemic H1N1 influenza (human and swine) isolates. Nine prototype H1N1 COBRA HA proteins were developed and tested in a virus-like particle (VLP) format for the elicitation of broadly-reactive, universal antibody responses, protection against viral challenges, and prevention of transmission in pre-clinical mouse and ferret models. H1N1 COBRA HA vaccines were designed to recognize H1N1 viruses within the last 10, 20, and 30 years, as well as COBRA vaccines that elicited antibody responses that recognized H1N1 viruses over the past 100 years, including modern pandemic H1N1 isolates. Four of the 9 H1N1 COBRA HA proteins (X-1, X-3, X-6, and P-1) had the broadest HAI and neutralization against a panel of 17 H1N1 viruses, with X-1 and P-1 COBRA HA proteins efficiently neutralizing and protecting ferrets against both seasonal and pandemic H1N1 challenges. COBRA-vaccinated animals had little or no detectable viral replication, less inflammation in the lungs, and reduced virus recovery in nasal washes. These vaccines were most effective in ferrets that were pre-immune to seasonal influenza viruses and subsequently vaccinated with H1N1 COBRA vaccines. Ferrets pre-immune to two seasonal H1N1 influenza viruses had HAI activity against only the pre-immune administered viruses. However, following H1N1 COBRA vaccination, these animals had HAI activity against all viruses in the panel, including pandemic H1N1 viruses (2009 to 2014), and completely protected against viral challenges. Furthermore, passively transferred immune serum from the COBRA HA VLP-vaccinated mice protected recipient animals more efficiently than immune serum from animals vaccinated with vaccines containing wild-type HA proteins. COBRA vaccines against H3N2 and other influenza A viruses, as well as influenza B, are underway. This is the first report describing the elicitation of universal, broadly-reactive, protective sterilizing immunity against H1N1 isolates using a COBRA-based HA strategy.

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Mucosal influenza vaccines

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Based on the understanding of the pathophysiology, we are currently investigating the protective immune responses against influenza viral infection. The 2009 pandemic of influenza virus highlighted the difficulty in predicting the subtype and strain of influenza viruses which cause a coming pandemic. This fear of an emerging pandemic of new influenza underscores the urgency of preparing effective vaccines to meet the pandemic. One mean to mitigate current concerns is to develop a flu vaccine that is functional against drift influenza viruses. In our current situation, in which we can not predict which strain will cause a pandemic, cross-protective immunity plays a particularly important role in preventing the spread of highly pathogenic influenza viruses.

Intranasal administration of a vaccine induces cross-protective secretory IgA (S-IgA) antibodies on the surface of nasal mucosa which is not induced by parenteral injection of the vaccine. S-IgA antibody in nasal mucus can, unlike serum IgG, prevent homologous and heterologous virus infection. However little is known about the quaternary structures and neutralizing potencies of the polymeric S-IgA antibodies in human. In this study, antibody responses induced by intranasal vaccination with a seasonal influenza viruses and Highly Pathogenic Avian Influenza virus (HPAIV) A(H5N1) Whole Inactibvated Virion (WIV) were measured in serum and nasal wash samples of healthy adult volunteers. Moreover the neutralizing ability of S-IgA antibodies and those structures were analyzed. The result showed that the intranasal vaccination of WIV can induce neutralizing antibodies both in serum and nasal mucus in human. Moreover, the polymeric S-IgA antibodies induced by intranasal vaccination play a pivotal role in cross-protection and neutralization of the virus.

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A universal influenza virus vaccine strategy based on the conserved stalk domain of the hemagglutinin

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Background: Influenza virus infections remain a significant cause of morbidity and mortality worldwide. Current vaccines show good efficacy against antigenically matched viruses by inducing strain specific antibodies against the membrane-distal globular head domain of the viral hemagglutinin, but fail to protect against drifted and pandemic strains. Due to the rapid antigenic drift of influenza viruses - especially in the globular head domain - these vaccines have to be re-formulated, generated and administered through a cumbersome and expensive process every year.The membrane-proximal stalk domain of the viral hemagglutinin exhibits a high degree of both sequence and structural conservation across influenza virus subtypes and monoclonal antibodies directed against this region typically show broad neutralizing activity. However, these antibodies are rare and usually not induced/boosted by regular seasonal vaccines. We hypothesize that a vaccine strategythat stimulates a robust immune response towards this region of the hemagglutinin could provide universal influenza virus protection.

Methods: We developed a universal influenza virus vaccine based on the conserved stalk domain of group 1 and group 2 hemagglutinins. By sequential vaccination of mice with chimeric hemagglutinin constructs that share the same stalk domain but have divergent head domains we were able to specifically boost broadly neutralizing antibody titers against conserved epitopes in the hemagglutinin stalk.

Results: Mice vaccinated with our constructs were protected from morbidity and mortality induced by infection with a panel of heterologous and heterosubtypic influenza A viruses. In the light of emerging viruses in Asia it is of note that our vaccination regimen also protected animals from H6N1 and H7N9 virus challenges and reduced lung titers upon H10 virus infection.In addition the chimeric HA based vaccination regimen also showed efficacy in ferrets, induced high titers of broadly reactive antibodies against divergent hemagglutinins from different subtypes and significantly reduced transmission in this model. Finally, we showed that stalk-reactive antibodies were boosted in individuals that received an H5N1 vaccine in clinical trials.This supports the hypothesis that exposure to hemagglutinins with divergent heads but conserved stalk induces such antibodies in humans.

Conclusions: The present data suggest that this vaccine strategy could be successfully developed in humans to provide broad influenza virus protection and enhance our pandemic preparedness.A universal influenza virus vaccine, which - similar to the ones developed for polio and measles viruses - requires a single or only a few immunizations, would represent a major advance towards the control of influenza worldwide.

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Adjuvants

Abstract not available at this time.

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Single replication influenza vaccine M2SR elicits long-lasting, cross-protective immunity

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Background: Influenza vaccines have remained virtually unchanged for decades. The most commonly used vaccine is the trivalent inactivated vaccine (TIV). Despite annual updating to match the circulating influenza strains, the effectiveness of TIV is only 60%. FluMist, a commercially available live flu vaccine, is effective in children but not adults. A vaccine with greater efficacy that also provides heterosubtypic protection would be considered a major transformation in the influenza vaccine market. Our M2SR (Single Replication) vaccine candidate, an influenza virus that does not express M2 protein, has achieved these characteristics in animal models and against multiple influenza subtypes (H3N2, H1N1 and H5N1).

Materials and Methods: Viruses were generated by standard influenza rescue techniques. H5N1 andH1N1M2SR viruses encode the hemagglutinin (HA) and neuraminidase (NA) from A/Vietnam/1203/2004 (VN1203,H5N1)andA/California/07/2009 (H1N1), respectively. Mice or ferrets were intranasally inoculated with H5N1 M2SR or H1N1 M2SR or mock-immunized. Sera were collected on days 7, 14, 21 post-inoculation. Animals were challenged with a lethal dose of VN1203 virus 20 weeks (mice) or 8 weeks (ferrets) later. HA-specific antibody responses were analyzed by ELISA or hemagglutination inhibition (HI) assays. Influenza-specific cell-mediated responses in bronchoalveaolar lavage or medial lymph nodes were analyzed by flow cytometry.

Results: All of the M2SR vaccinated mice and ferrets survived lethal VN1203 challenge whereas none of the mock-immunized group survived. H5N1 M2SR vaccinated animals induced high levels of IgG and IgA in both serum and lung wash. These mice also displayed high HI titers whereas the H1N1 M2SR, and mock-immunized groups did not display any HI activity. The H5N1 M2SR vaccinated animals did not have any detectable challenge virus in any organ indicating sterile immunity. In contrast, virus was recovered from multiple organs in the mock-immunized mice (systemic infection).

Conclusions: A single dose of M2SR vaccine induced long-lasting cross-protective immunity against lethal H5N1 challenge in mice and ferrets (supported by NIAID Contract # HHSN272201000031I). Preliminary results suggest that multiple cross-reactive immune responses contribute to the breadth of protective immunity afforded by M2SR against different influenza virus subtypes.

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Cell culture based influenza vaccine

In Japan, influenza vaccines have been manufactured using embryonated chicken eggs as virus culture substrate material for more than 40 years. Although the egg system has strengths, such as high viral growth, initial purity and low adventitous agents, there are also several issues. Firstly, viral isolation in eggs is becoming difficult, especially for H3, leading to mismatches between the vaccine and the circulating strain. Secondly, antigenic changes are evident upon serial passaging in eggs, especially for H3. Finally, there is low vaccine manufacturing flexibility in a pandemic situation, when a pandemic is caused by a highly-pathogenic avian influenza virus.

After requests and support from the government, three vaccine manufacturers have developed cell culture derived H5N1 vaccines. At Kaketsuken, we are using full suspension EB66 cells for the substrates, in collaboration with GSK. The other two companies are using adherent Vero and MDCK cells. Thanks to this national project, a pandemic vaccine for almost the entire Japanese population can be produced 6 months after a vaccine strain is established.

In our clinical studies, it was shown that the vaccine elicited homologous HI antibody responses that exceeded CHMP criteria after 2 doses. Marked increase of neutralizing antibody after 2 doses was also confirmed. Antibody induced by the vaccine was cross-reactive against drift variants. Regarding safety, no incidence of serious adverse events or potential immune-mediated diseases related to KD-295. Based on frequency and intensity of adverse events, the reactogenicity and safety data obtained up to Day 201 suggest an acceptable safety profile.

To maintain the cell culture facilities in inter-pandemic periods, and to address the issues related to the egg-derived seasonal vaccine, we have to accelerate the seasonal vaccine development using the cell culture platform. For this end, the selection of good candidate vaccine viruses (CVV) is very important. As serial passaging in eggs results in antigenic changes, these CVV should be cell culture isolates. Currently, a scheme to distribute MDCK isolates for the CVV is being established by the IFPMA and WHO influenza collaborative centers.

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Status and Clinical Development of Next Generation Adjuvants For Respiratory Diseases

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No defined, synthetic adjuvants are components of approved vaccines. However, safe and effective adjuvants for prophylactic and therapeutic vaccine use are emerging from the identification and optimizing formulations of small molecules. Effectively engaging macrophages and dendritic cells (DC), leading to T cell responses is essential for developing a new generation of T cell vaccines (e.g. tuberculosis, malaria, HIV), as well as for improving the quality and duration of antibody responses (influenza, HIV). The most advanced approaches to new adjuvant development consist of using TLR ligands (TLRL), to provide synergism between the formulation and the TLRL. Based on the success of the endotoxin derived natural product, MPL®, a TLR4L, a number of synthetic molecules have been developed and formulated into promising adjuvants. Position, number, and length of acyl chains present in the TLR4L all influence responses by human antigen-presenting cells (APC). Formulation dramatically influences the nature of the immune response induced. We have developed formulations of our lead TLR4L, GLA, and have evaluated a variety of these, including oil/water emulsions, micellar, niosomal, alum-adsorbed, and liposomal, in clinical trials and in a variety of preclinical models. When properly formulated, GLA-based adjuvants enhance Th1 type responses in both mice and humans, as well as induce more rapid immune responses against viral pathogens. Thus, it appears that selective molecular synthesis and formulation may lead to a new generation of TLR4L- based adjuvants with improved qualities over natural products. Clinical data on the use of next generation adjuvants for influenza and tuberculosis vaccines will be presented.

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Comparison of antiviral activity between IgA and IgG specific to influenza virus hemagglutinin: Increased potential of IgA for heterosubtypic immunity

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Influenza A viruses of 16 hemagglutinin (HA; H1-H16) subtypes are maintained in the waterfowl reservoir. Viruses of H1, H2, and H3 subtypes are known to have caused pandemics in humans, and the emergence of new pandemic viruses of other HA subtypes has been a public health concern. Although both IgA and IgG antibodies are known to play important roles in protection against influenza virus, the contribution of these antibodies to the cross-protective heterosubtypic immunity is not fully understood. To compare in vitro antiviral activities of monoclonal IgA and IgG recognizing the same epitope on the HA molecule, polymeric IgA-producing hybridoma cells were subcloned from those originally producing S139/1, an HA-specific monoclonal IgG that was generated against a virus strain of the H3 subtype but had cross-neutralizing activities against the H1, H2, H13, and H16 subtypes. S139/1-IgA and S139/1-IgG were used to directly compare antiviral activities between the isotypes. Both S139/1-IgA and S139/1-IgG similarly bound to the homologous H3 virus in an enzyme-linked immunosorbent assay, and there were no significant differences in their hemagglutination-inhibiting and neutralizing activities against the H3 virus. In contrast, S139/1-IgA showed remarkably higher cross-binding and antiviral activities against viruses of the heterologous subtypes than S139/1-IgG. It was also noted that S139/1-IgA, but not S139/1-IgG, drastically suppressed the extracellular release of the viruses from infected cells. Electron microscopy revealed that S139/1-IgA deposited newly produced viral particles on the cell surface, most likely by tethering the virus particles. On the other hand, we found that both subcutaneous and intranasal immunizations induced antibody responses to multiple HAs of different subtypes, whereas IgA was not detected remarkably in mice immunized subcutaneously. As expected, no heterosubtypic neutralizing activity was detected by a standard neutralization test in which viruses were mixed with antibodies prior to inoculation into cultured cells. Interestingly, however, a remarkable reduction of plaque formation and extracellular release of the heterologous virus was observed when infected cells were subsequently cultured in the presence of HA-specific cross-reactive IgA but not IgG antibodies. Taken together, these results suggest that the majority of HA-specific cross-reactive IgG and IgA antibodies produced by immunization do not block cellular entry of viruses, but cross-reactive IgA has greater potential to prevent influenza A virus infection than IgG, likely due to its multivalency and increased avidity, and that this advantage may be particularly important for heterosubtypic immunity.

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Pathogenic Mechanisms of the Emerging Coronaviruses

Coronaviruses cause high morbidity and mortality in humans and animals. Over the past decade, two highly pathogenic human coronaviruses have emerged from bats and other intermediate hosts, designated Severe Acute Respiratory Coronavirus (SARS-CoV) and Middle East Respiratory Coronavirus (MERS-CoV). The emergence mechanisms of coronaviruses is associated with zoonotic virus recognition of ortholog receptors across two or more species, and the molecular mechanisms driving cross species transmission events and improved animal model development will be discussed. The emerging respiratory cornaviruses cause acute respiratory distress syndrome (ARDS), a severe end stage lung disease associated with 50% mortality rates. Using robust animal models, we are studying the genetic and molecular mechanisms governing host susceptibility to highly pathogenic respiratory virus infection, using systems biology, candidate gene approaches and unbiased genetic screens. We will demonstrate novel and overlapping strategies of ISG manipulation following high/low path virus infection, and discuss mechanisms of ISG control. Using these data and an unbiased candidate gene approach, we identify target ISGs and other host genetic pathways that mediate protective or pathogenic roles following SARS-CoV and MERS-CoV infections.

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Predicting Epidemic Strains of Influenza Viruses

Abstract not available at this time.

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Broad Neutralization of Influenza Viruses and Implications for a Universal Vaccine and Therapy

The major surface antigen, the hemagglutinin (HA), of influenza virus is the main target of neutralizing antibodies. However, until recently, most antibodies were thought to be strain-specific and protect only against highly related strains within the same subtype. In the few years, a number of antibodies have been isolated that are much broader and neutralize across subtypes and groups of influenza A, as well as influenza B, viruses through binding to functionally conserved sites. We have determined structures of several broadly neutralizing antibodies with the HA and show their epitopes map to highly conserved sites on the HA fusion domain (stem) in both influenza A (1-3) as well as influenza B (4) viruses. We have also investigated antibodies that bind more broadly to the receptor binding site and protect against different strains and subtypes (e.g. 4,5). The identification and characterization of the epitopes and mode of binding of these antibodies provide exciting new opportunities for structure-assisted vaccine design as well as for design of therapeutics that afford greater protection against influenza viruses.

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Human Infections by Avian Influenza Viruses – Mechanism Study and Potential Application to Increase Viral Yields in Cell-Based Vaccine Productions

Updated abstract not available at this time.

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Designing Clinical Trials for Novel ARI Vaccines: Primum Non Nocere

Background and Comments. Acute viral respiratory viral infections (ARIs) cause significant morbidity and mortality worldwide in all age groups and populations. Among these, influenza, respiratory syncytial virus (RSV) and emerging coronaviruses (SARS and MERS) pose daunting challenges related to vaccine development.

Antigenic variation of influenza viruses requires annual reformulation of vaccines. Therefore, vaccines capable of eliciting broadly neutralizing antibodies or targeting novel conserved antigens are desirable. Ideally such vaccines would not require annual reformulation and/or annual immunization. Poor immunogenicity of candidate inactivated avian influenza virus vaccines indicates the need for inclusion of novel adjuvants, which may be associated with acute toxicity or long-term sequelae. Prepandemic vaccines may not match the ultimate pandemic virus, further emphasizing the need for vaccines that stimulate more broadly cross-reactive responses.

RSV has its greatest impact in infants and young children. Development of vaccines has been hampered by the observation that formalin-inactivated vaccines elicited immunopathological responses in infants. The pathogenesis of these reactions has not been determined, and the pace of vaccine development was slowed following this observation. Novel strategies, including maternal immunization for passive protection of the infant; and cautious assessment in seropositive (previously RSV- infected) children followed by seronegative (RSV-naïve) children, will be necessary to evaluate candidate RSV vaccines.

There is also concern regarding the potential for eliciting immunopathological responses following immunization with candidate SARS vaccines based on animal models.

Designing clinical trials to assess safety and efficacy of candidate novel ARI vaccines will be discussed. Selected examples of clinical trials of novel ARI vaccines will be presented. Trial design is determined by prior experience, the nature of the product being tested and the need for identification of predictors of immune response and correlates of protection (COP). Careful subject screening for health +/- susceptibility to infection with vaccine +/- vector may be necessary. Subjects may need to be sequestered when evaluating candidate live vaccines. Common adverse events (AEs) typically are identified in phase I-phase II trials, whereas rarer AEs may not be identified until phase III efficacy or post-marketing phase IV trials are undertaken. Careful monitoring of vaccinated individuals for the occurrence of naturally–occurring infection may be necessary, even in the early phases of development. Long-term follow-up of vaccinated individuals and identification of new COP including in-depth analyses of antibody and cell mediated immune responses may be necessary in order to establish the safety and efficacy of novel vaccines for prevention of ARI

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ARI Vaccines Post Licensure: What Do We Expect and What are We Looking For?

Abstract not available at this time.

厚生労働科学研究委託費（地球規模保健課題推進研究事業（国際医学協力研究事業））
委託業務成果報告（業務項目）

エイズの研究・米側との専門協議

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研究要旨

HIV 感染症の制圧はグローバルな視点で取り組むべき重要課題であり、多様性に富む HIV の流行抑制に向けて世界各地域の流行状況を把握することは重要である。本研究では、特にアジアにおける HIV 感染流行状況の把握等を目指し、米国研究者との情報交換を進めることとした。平成 26 年度は、台北で日米エイズパネル会議を開催し、HIV に対する免疫反応、宿主因子とウイルス多様性、構造ウイルス学および潜伏感染と治療をテーマとして、アジアの研究者も含め情報交換を行った。

A. 研究目的

世界三大感染症の一つである HIV 感染症は、慢性持続感染を呈し、その結果エイズ発症にいたる致死感染症である。その制圧は、グローバルな視点で取り組むべき重要課題であり、世界各地域の流行株を把握することは、多様性に富む HIV の感染拡大抑制に向けた取り組みにおいて重要な基盤情報となる。

HIV ゲノム塩基配列の解析研究においては、まず、主に env 領域の解析に基づいて各種サブタイプが同定されてきた。さらに、1990 年代後半の抗 HIV 薬治療導入以降は、主に pol 領域の解析に基づく薬剤耐性変異の同定も進められてきた。

一方、細胞傷害性 T 細胞（CTL）反応は HIV 複製抑制に中心的役割を担っており、その標的抗原エピトープを提示する HLA(ヒト白血球抗原) クラス I の遺伝子型は、HIV 感染病態に大きな影響をおよぼすことが知られている。また、各々の HLA 遺伝子型に相関する変異（HLA 関連変異）の多くは CTL 逃避変異を反映するものであるが、各 HLA アレル頻度は人種間で大きく異なるため、世界各地域で多様な HIV ゲノムと多様な宿主 HLA 遺伝子型の相互作用のもと、多様な HLA 関連変異の伝播が生じていると考えられる。

本研究は、HIV 感染症制圧を目指すグローバルな視点での取り組みに向け、米国研究者との情報交換を進めるものである。特にアジアにおける HIV 感染流行状況の把握に向けて各地域の HLA 遺伝子型と HIV ゲノム情報の整備を目指すこととした。平成 26 年度は、HIV に対する免疫反応、宿主因子とウイルス多様性、構造ウイルス学および潜伏感染と治療をテーマとして、情報交換・議論を行った。

B. 研究方法

主にベトナム HIV 感染者のウイルスゲノム解析およびサルエイズモデルにおけるウイルス CTL 逃避変異情報を収集した。2015 年 1 月 26 日−27 日の EID（Emerging Infectious Diseases）会議に引き続き、1 月 28 日−29 日に日米エイズパ

ネル会議を台北にて開催した。

（倫理面への配慮）

ヒトサンプルを用いた研究ならびに動物実験については、関連する指針に基づき、所属機関の倫理委員会および動物実験委員会の審査をうけ、その承認を得てから開始した。

C. 研究結果

HIV 感染者におけるウイルスゲノム塩基配列情報およびサルエイズモデルにおけるウイルス CTL 逃避変異情報を蓄積した。日米エイズパネル会議では、表１のように、HIV に対する免疫反応、宿主因子とウイルス多様性、構造ウイルス学および潜伏感染と治療の４つのテーマに関して、日米およびアジアの研究者あわせて 23 名の研究発表が行われ、議論が行われた。HIV に対する免疫反応のセッションでは、抗 HIV 抗体に関する最新の知見が得られ、宿主因子とウイルス多様性のセッションでは、特にアジアにおけるウイルス流行状況に関する最新の情報が得られた。構造ウイルス学のセッションでは、ウイルス複製抑制因子に関する研究進展状況を共有することができ、潜伏感染と治療に関しては、治療薬投与量軽減に結びつく知見と治癒に向けた取り組みに関する最新情報が得られた。

D. 考察

日米エイズパネル会議において、アジアの HIV 流行状況の詳細について情報を共有することができた。治療薬投与量軽減に関する知見等、HIV 感染者治療に直結する情報に加え、感染拡大抑制や感染者治癒に向けた長期的視点に基づく取り組みに結びつく情報も交換することができた。このように、アジア研究者も含めた日米研究者間の持続的情報交換・共有の重要性が再認識された。

E. 結論

HIV 感染症の制圧を目指すグローバルな視点での取り組みに向け、特にアジアにおける HIV 感染流行状況の把握等を目指し、米国研究者との情報交換を進めることとした。平成 26 年度は、台北で日米エイズパネル会議を開催し、HIV に対する免疫反応、宿主因子とウイルス多様性、構造ウイルス学および潜伏感染と治療をテーマとして、アジアの研究者も含め情報交換を行った。

F．研究発表

１　論文発表

(1) Nomura T, Yamamoto H, Takahashi N, Naruse TK, Kimura A, Matano T. Identification of SIV Nef CD8$^+$ T cell epitopes restricted by a MHC class I haplotype associated with lower viral loads in a macaque AIDS model. Biochem Biophys Res Commun 450: 942-947, 2014.

２　学会発表

該当無し。

G．知的財産権の出願・登録状況

該当無し。

表１．日米エイズパネル会議発表者リスト（2015年１月）

Opening Remarks by Tomas Hope (Northwestern University)

Session A. Anti-HIV Responses

Yen Li	NIH	Recent Advances in AIDS Vaccine Research
Dan Barouch	Harvard	Ad26/MVA and Ad26/Protein Mosaic Vaccines for HIV-1
Ai Kawana-Tachikawa	University of Tokyo	Epigenetic repression of IL2 expression in senescent CD4+ T cells in chronic HIV-1 infection
Margie Ackerman	Dartmouth College	Modeling antibody effector function
Tomas Hope	Northwestern University	Harnessing antibody interactions with mucins to enhance HIV vaccine function
Kazuhisa Yoshimura	NIID	Impact of the drug-escaped HIV envelope mutations on susceptibility to neutralizing antibodies

Session B. **Host Factors and Viral Diversity**

Yi-Chun Lo	Taiwan CDC	National Trend and Characteristics of Acute Hepatitis C among HIV-Infected Individuals: a Case-Control Study — Taiwan, June 2001–November 2014
Jyh-Yuan Yang	Taiwan	HIV Drug Resistance Survey among treatment naïve patients in Taiwan, 2010 to 2013
Tatsuo Shioda	Osaka University	Host factors in the pathogenesis of HIV infection
Ed Campbell	Loyola University	HIV_1 uncoating is faciliated by dynein and kinesin-1
Li-Min Huang	National Taiwan University	Large Isoform of Mammalian Relative of DnaJ is a Major Determinant of Human Susceptibility to HIV-1 Infection
Fu-Tong Liu	Academia Sinica	Galectin-3
Baek Kim	Emory	Roles of Host SAMHD1 Protein in HIV-1 Biology in Macrophages

Session C. **Structural Virology**

Zene Matsuda	University of Tokyo	Insertional mutagenesis of HIV-1 envelope glycoprotein with variant GFP
Dmitri Ivanov	University of Texas Health Science Center	Structural biology of TRIM5α-mediated retroviral restriction
Barbie Ganser-Pornillos	University of Virginia	Recognition of the HIV capsid by the restriction factor TRIM5α
Akifumi Takaori-Kondo	Kyoto Univ	Functional interaction between APOBEC3 and HIV-1 Vif

Session D. Long-term Therapy & HIV Persistence/ Intervention Towards a Cure

Kiat Ruxrungtham	Chulalongkorn Univ, Bangkok	Optimizing Antiretroviral therapy -Asia perspective
Shu-Wen Lin	National Taiwan University Hospital	Association between plasma concentration of cotrimoxazole and its adverse events in HIV patients with Pneumocystis jiroveci pneumonia
Yi-Ming Arthur Chen	Kaohsiung Medical University	Clinical and Virological Characterization of CRF07_BC infection
Yoshio Koyanagi	Kyoto Univ	Genome-editing technologies for excision of HIV provirus
Yorifumi Satou	Kumamoto Univ	Regulatory mechanism of proviral transcription
Deborah Persuad	Johns Hopkins University	Early Therapy and HIV Persistence in Perinatal Infection

Closing Remarks by Tetsuro Matano (University of Tokyo)

厚生労働科学研究委託費（地球規模保健課題推進研究事業（国際医学協力研究事業））
委託業務成果報告（アジア地域にまん延している疾病に関する研究）

コレラ・細菌性腸管感染症の研究・米側との専門協議に関する研究

担当責任者　西渕 光昭　京都大学東南アジア研究所・教授

分担研究要旨

本研究の第I部では、担当責任者が、現在アジアで重要な腸管感染症の代表として、魚介類の喫食を介して発生する腸炎ビブリオ感染症および牛肉の喫食を介して発生する腸管出血性大腸菌感染症予防のために必要な食材（魚介類・牛肉）の簡便かつ高感度な検査法の確立について報告する。第II部では、平成27年1月に米国フロリダ大学で開催された日米医学協力コレラ・細菌性腸管感染症部会(以下コレラ部会と略す)合同部会の概要（日本側発表者の発表内容を含む）を紹介する。

A．研究目的

A-I. 食材中の腸炎ビブリオおよび腸管出血性大腸菌の病原性株の簡便・高感度検査法の確立

1996 年頃からアジアから世界に広がった新型腸炎ビブリオ感染症は、わが国ではほぼ征圧できたが、世界の他の地域（特に熱帯地域の発展途上国）ではまだ猛威を振るっている。腸管出血性大腸菌感染症は世界の先進国（アジアでは日本）を中心に、多発しているが、主たる原因菌の血清型は、0157 のみならず、それ以外の O 血清型以外が次第に顕著になりつつある。最近の食材のグローバル化と貿易量の増加を考慮すると、これらはアジア諸国のみならず全世界を巻き込んだグローバルヘルスに関する重大な問題の１つと考えるべき状態にある。これに対して、WHO と FAO 傘下にあるコーデックス委員会が中心となって、食材の取り扱いから消費に至るまでの衛生規範を厳しくするとともに、定量リスクアセスメントの結果に基づく規制値（検出限界値）を決定する evidence-based medicine (EBM) 的アプローチを推進している。しかし、後者に関しては、食材中の定量データが、かり不足しているので、直ちに実施できないという状態にある。これを解決するために、担当責任者は、過去の研究を継続し、上記 2 菌種に属する病原性菌株を世界のどのような国でも実施できる簡便かつ高感度な世界標準食品検査法を確立することが、本研究の目的である。

A-II. 平成 26 年日米医学協力コレラ部会合同部会の概要

アジアの人々の腸管感染症による健康被害の改善を目標とする日米医学協力コレラ部会合同部会は、米国主催時の予算獲得問題と臨地研究の重視という観点から、日米双方の合意のもと、最近の米国主催時の開催地は、インドのコルカタ、(平成 23 年度)、バングラデッシュのダッカ（平成 25 年度）と臨機応変的変更があった。26 年度は日本側パネルが合同会議を担当する順番であった。米国内での合同会議が 2 度続けて米国で開催されなかったことに対して、評価委員から日本側若手部会メンバーへの刺激が必要であるという辛口のコメントがあった。

この点を改善するために、26年度は日本側も、米国側の了解のもとに、米国開催という歴史上異例の対応策をとった。形式上は日本が責任国として、西渕の長年の友人であるフロリダ大学新興感染症病原体研究所所長J. Glenn Morris, Jr.教授にお願いして、ローカルホストになっていただいた。フロリダのゲインズビルに残るアメリカらしさの他に、会議内容にも新たな試みを盛り込んで、米国側と協働して、リフレッシュした合同会議を企画する事を目的とした。

B．研究方法

B-I. 食材中の腸炎ビブリオおよび腸管出血性大腸菌の病原性株の簡便・高感度検査法の確立

いずれの菌種を対象とする検査法においても、MPN (most probable number) 法に基づく定量検出へ繋げる検査法として、液体培地を用いた特異的増菌培養法をベースにして、標的菌体最外部表面抗原に対する免疫磁気ビーズを用いた分離（IMS）法および主たる病原遺伝子を標的とするLAMP法を組み合わせて、検査法に簡便性よび特異性を導入した。

（倫理面への配慮）：特に対象となる研究内容は含まれていない。

B-II. 平成26年日米医学協力コレラ部会合同部会の概要

平成26年度は日本側も、米国側の了解のもとに、米国開催という歴史上異例の対応策をとった。形式上は日本が責任国として、西渕の長年の友人であるフロリダ大学新興感染症病原体研究所所長J. Glenn Morris, Jr.教授にお願いして、ローカルホストになっていただいた。フロリダのゲインズビルに残るアメリカらしさの他に、会議内容にも新たな試みを盛り込んで、米国側と協働して、リフレッシュした合同会議を企画する事を目的とした。

（倫理面への配慮）：特に対象となる研究内容は含まれていない。

C．研究結果

C-I. 食材中の腸炎ビブリオおよび腸管出血性大腸菌の病原性株の簡便・高感度検査法の確立

腸炎ビブリオに関しては、既知のK抗原すべてを標的とするIMS法および*tdh*遺伝子を標的とするLAMP法を組み合わせて、簡便性よび特異性を確保し、MPN法に基づく定量検査法も開発した（論文2）。この方法を、日本およびタイ国において市販されている二枚貝の検査に適用したところ、後者において高濃度の標的菌を含むサンプルの一部において、菌濃度の過小評価が認められた以外は良い結果が得られた。前者についても低濃度の標的菌を含むサンプルでも高感度検出が可能になったことが検証できた（論文2）。さらに、その後の研究において、タイ国の二枚貝サンプル中の菌濃度の過小評価の問題は、IMS法の増菌培養液への適用条件を改良することで改善できた。この改良法を日本で販売されている二枚貝サンプルの検査に適用しても問題は発生しなかったので、現段階でこの改良法が世界標準検査法に最も適していると判断した（Escalante-Maldonado et al., 投稿中）。

腸管出血性大腸菌に関しては、志賀毒素遺伝子（*stx*）を保有する大腸菌（STEC）菌株の中で、主要な腸管出血性大腸菌菌株