High Yield Production of Influenza A and B Virus in Madin Darby Canine Kidney (MDCK) Cells with Stable Knockdown of IRF7-like Gene

Masato Tashiro*, Itsuki Hamamoto, Norio Yamamoto

Influenza is a serious public health problem that causes a contagious respiratory disease as seasonal epidemics and pandemic influenza. Vaccination is the most effective strategy to reduce transmission and prevent influenza disease burden. In recent years, cell-based vaccines have been developed with continuous cell lines such as Madin-Darby canine kidney (MDCK) and Vero cells. However, wild-type influenza and egg-based vaccine seed viruses will not grow efficiently in these cell lines. Therefore, improvement of virus yield is stronglyrequired for development of vaccine seed viruses and cell-based influenza vaccine production. The aim of our research is to develop novel MDCK cells supporting highly efficient influenza virus propagation in order to expand the capacity of vaccine production. In this study, we screened a human siRNA library that involves 78 target molecules relating to three major signaling pathways oftype I interferon (IFN) to identify genes knockdown of which by siRNA significantly enhanced the production of influenza virus A/Puerto Rico/8/34 in A549 cells. The siRNAs targeting 23 candidate genes were selected for a second screening pass in MDCK cells. We examined the effects of knockdown of target genes on the viral production using newly designed siRNAs based on our data of sequence analysis. Knockdown of the expression of a canine gene corresponding to human IRF7 (IRF7-like gene) by siRNA increased the efficiency of viral production in MDCK cells through an unknown process that includes the mechanisms other than inhibition of IFN-a/b induction. Furthermore, the yield of influenza A and B viruses so far tested was increased sufficiently in MDCK cells, which were transduced stably with the lentiviral vector for expression of short hairpin RNA (shRNA) against IRF7-like gene. Wepropose therefore that modified MDCK cells with lowered level of IRF7-like gene expression could be useful for not only increasing the capacity of vaccine production but also facilitating the process of vaccine seed virus isolation from clinical specimens.

Analysis of protective immune responses after intranasal administration of an inactivated whole-virion influenza vaccine in human

Hideki Hasegawa
Department of Pathology, National Institute of Infectious Diseases

Intranasal administration of vaccine can induce secretory IgA (S-IgA) antibodies on the surface of nasal mucosa. S-IgA antibody in nasal mucus is superior to IgG antibody in serum for the protection against infection at the infection site. Moreover S-IgA antibodies are cross protective against variant influenza viruses. In this study, the efficacy of intranasal administration of an inactivated whole-virion vaccine was investigated among healthy adult volunteers.

Fifty volunteers received the intranasal vaccination of an inactivated whole-virion influenza vaccine twice at 3-week intervals, that contains 45ug of HA molecules per dose. Serum and nasal wash (NW) were collected at each time point, and assayed for neutralizing antibody (NT) titer and hemagglutination inhibition (HI) titer. The high levels of NT and HI titer were detected not only in the serum but also in the NW after the intranasal vaccination for the first time. Although these titers in sera were well correlated with IgG antibody titer, those in NWs were correlated with S-IgA antibody titer rather than IgG antibody titer. The neutralizing antibody titer was well correlated with the HI titer both in the serum and nasal wash. Cross-protective neutralizing antibodies were analyzed against heterologous viruses. These results suggest that the S-IgA antibody induced by intranasal inactivated vaccine could play an important role in direct neutralization of the viruses at the infection site in human.

A novel *Escherichia coli* derived pandemic influenza vaccine that induces neutralizing antibodies and Th1 responses in mice and ferrets

David A. G. Skibinski[1,2], Brendon J. Hanson[3], Yufang Lin[2], Veronika von Messling[4], Andrea Jegerlehner[5], Jason Boon Sern Tee[3], De Hoe Chye[3], Steven K. K. Wong[3], Amanda A. P. Ng[1,2], Hui Yin Lee[2], Bijin Au[2], Bernett T. K. Lee[2], Lucia Santoso[6], Veronica Diermayr[7], Michael Poidinger[2], Anna-Marie Fairhurst[2], Alex Matter[6,7], Martin F. Bachmann[5], Philippe Saudan[5], John E. Connolly[1,2]#

[1] A*STAR Program in Translational Research on Infectious Disease
[2] Singapore Immunology Network (SIgN), Agency for Science, Technology and Research (A*STAR), 8A Biomedical Grove, Immunos Building, Level 3, Biopolis, Singapore 138648
[3] DSO National Laboratories, 27 Medical Drive, Singapore 117510
[4] INRS-Institut Armand-Frappier, University of Quebec, Laval, QC, Canada
[5] Cytos Biotechnology AG, Zurich-Schlieren, Postfach 8952, Switzerland
[6] Experimental Therapeutics Centre, Experimental Therapeutics Centre (ETC), Agency for Science, Technology and Research (A*STAR), 31 Biopolis Way, Nanos Level 3, Singapore 138669
[7] D3 (Drug Discovery and Development), BMSI, 31 Biopolis Way, #01-02a Nanos, Singapore 138669

Influenza pandemics can spread quickly and cost millions of lives; the 2009 H1N1 pandemic highlighted the shortfall in the current vaccine strategy and the need for an improved global response in terms of shortening the time required to manufacture the vaccine and increasing production capacity. Here we describe the comprehensive pre-clinical assessment of a novel 2009 H1N1 pandemic influenza vaccine based on the *E. coli*-produced HA globular head domain covalently linked to virus-like particles derived from the bacteriophage Qβeta. Immunization of mice, induced antibody titers comparable to the licensed 2009 H1N1 pandemic vaccine Panvax, and significantly reduced viral titers in the lung following challenge with 2009 H1N1 pandemic virus. While Panvax failed to induce marked T cell responses, the novel vaccine stimulated substantial antigen-specific interferon-γ production in splenocytes from immunized mice, alongside enhanced IgG2a antibody production. In ferrets the vaccine elicited neutralizing antibodies, and reduced morbidity following challenge and lowered viral titer in nasal lavages. A clinical assessment of the gH1-Qβeta vaccine, planned for this year, will reveal how these encouraging pre-clinical data translate into the human setting.

NIAID Perspectives on Universal Influenza Vaccine Development

Rachelle Salomon, PhD

Influenza, SARS, & Related Viral Respiratory Diseases Section
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases
NIAID/NIH/DHHS
Bethesda, MD USA

The National Institute of Allergy and Infectious Diseases (NIAID) is the second largest institute of the National Institutes of Health (NIH) charged with supporting and conducting research on infectious diseases and diseases of the immune system. This institute is unique in that it has a dual mandate. The first responsibility is to cultivate a basic and applied research in microbiology, infectious diseases, immunology, and immune-mediated diseases. Secondly, NIAID has the duty to respond rapidly to new and emerging diseases threats. In light of this dual mandate, the Respiratory Diseases Branch (RDB) of the Division of Microbiology and Infectious Diseases (DMID) has continuously advanced vaccine development during the recent outbreaks of H5N1 influenza, SARS coronavirus and 2009 pandemic H1N1 influenza. This strategy has informed the influenza vaccine development efforts and led to many significant basic and clinical scientific advances. One of the lessons learned is that there is a need to development more broadly protective or universal influenza vaccines. NIAID is currently supporting several efforts towards universal influenza vaccines.

Developing a Universal, Broadly Reactive Influenza Virus Vaccine.

Corey J. Crevar[1], Brendan M. Giles[1], Donald M. Carter[1], Stephanie J Bissel[2], Clayton A Wiley[2], and Ted M. Ross[1,3]

Center for Vaccine Research[1] and Departments of Pathology[2] and Microbiology & Molecular Genetics[3], School of Medicine, University of Pittsburgh, Pittsburgh, PA, USA

Background: In this study, we have engineered an influenza virus-like particle (VLP) that contains a synthetic, consensus-based HA molecule based upon a new methodology, computationally optimized broadly reactive antigen (COBRA).
Methods: Three COBRA H5N1 HA proteins have been engineered based upon 1) human clade 2 H5N1 sequences, 2) human and avian clade 2 sequences, and 3) all H5N1 influenza sequences. COBRA vaccines were generated for H1N1 based upon seasonal and novel H1N1 sequences. Each HA retained the ability to bind the appropriate receptors, as well as mediate particle fusion. Non-infectious recombinant VLP vaccines using the COBRA HA molecules from were generated from a mammalian expression system. COBRA VLP vaccines were administered to mice, ferrets, and cynomolgus macaques and the humoral immune responses were compared to those induced by VLPs containing an HA derived from a primary viral isolate or a mixture of primary isolates.
Results: Using a single vaccination at 0.6ug HA dose with an adjuvant, all animals vaccinated with COBRA clade 2 HA H5N1 VLPs had protective levels of HAI antibodies to a representative isolate from each subclade of clade 2, but lower titers against other clades. The addition of avian sequences from other clades expanded breadth of HAI antibodies to the divergent clades 1, 4, and 7. Furthermore, all vaccinated animals were completely protected from challenge with the highly pathogenic clade H5N1 viruses. Little damage was observed in the lungs of COBRA VLP vaccinated animals as detected by H&E staining and little virus could be detected by immunohistochemistry staining of lung tissues or by viral plaque assay of lung homogenates.
Conclusion: This is the first report describing the use of a H5N1 VLP vaccine containing a synthetic HA antigen. The results show that the COBRA HA H5N1 VLPs elicit broad humoral immunity and is an effective influenza vaccine against HPAI virus in multiple animal models.
This work was supported by an NIH/NIAID award U01AI077771 to TMR. This work is also supported by a collaborative research grant from PATH Vaccine Solutions.

What drives the diversification of H5N1 influenza virus?

Gavin JD Smith

Duke-NUS Graduate Medical School, 8 College Road, Singapore 169857; and Duke Global Health Institute, Duke University, Box 90519, Durham, North Carolina 27708

The Asian highly pathogenic avian influenza H5N1 virus was first detected in the goose population of Guangdong, China in 1996. The viruses in this lineage are unique in their ecological success, demonstrating an extremely broad host range and becoming established in poultry over much of Asia and in Africa. H5N1 viruses have also diverged into multiple clades and subclades that generally do not cross neutralize, which has greatly confounded control measures in poultry and pre-pandemic vaccine strain selection. In addition to causing unprecedented economic losses, the long-term presence of H5N1 virus in poultry and its frequent introductions to humans continue to pose a significant pandemic threat. Here I provide a summary of the genesis and evolution of H5N1 viruses with particular reference to the factors that have contributed to their continued diversification.

Incidence of childhood pneumonia and serotype distribution in Streptococcus pneumoniae isolates after introduction of pneumococcal conjugate vaccine in Japan

Naruhiko Ishiwada[1)], Sachiko Takahashi[2)], Junko Tanaka[2)], Haruka Hishiki[2)], Chang Bing[3)]

1) Division of Control and Treatment of Infectious Diseases, Chiba University Hospital, Chiba, Japan
2) Department of Pediatrics, Chiba University Graduate School of Medicine, Chiba, Japan
3) Department of Bacteriology, National Institute of Infectious Diseases, Tokyo, Japan

Streptococcus pneumoniae is a common etiological agent of community-acquired pneumonia (CAP) in children. The 7-valent pneumococcal conjugate vaccine (PCV7) is reported to decrease the incidence of CAP among children. However, little is known about the rate of pneumonia attributable to *S. pneumoniae* and their serotypes.

To determine the incidence of CAP among Japanese children prior to the introduction of PCV7, we counted the number of children hospitalized with CAP between 2008 and 2009 in Chiba city, Japan. The annual incidence of hospitalized CAP among children <5 years old was 17.6 episodes per 1000 child-years. Among 626 CAP episodes, S. pneumoniae were dominantly isolated from 92 sputum samples and five blood samples. The most common serotypes were 6B, 23F and 19F. The coverage rates of PCV7 were 66.7% and 80% in sputum samples and blood samples, respectively. MLST analysis revealed 37 STs. Furthermore, 54.1% of the sputum isolates and 40% of the blood isolate were related to the international multidrug-resistant clones.

PCV7 was introduced in Japan in February 2010. Fortunately, Japanese government decided to subsidize for the vaccination of PCV7 in February 2011. Most of the children less than 5 years of age were able to vaccinated PCV7 free of charge.

To evaluate the effectiveness of PCV7 program, we conducted a prospective surveillance study of CAP in Chiba city between April 2012 and September 2012. During the study period, CAP caused 266 episodes of children being hospitalized. Three patients were diagnosed with pneumococcal bacteremia combined with CAP. *S. pneumoniae* was culture-dominantly identified in 17 sputum samples. The coverage rates of PCV7 were 26.7% and 0% in sputum and blood isolates, respectively. PCV7 vaccination rate of the children with CAP was 74%. Pneumococcal pneumonia, especially PCV7 related one were decreased 2 years after start of PCV7 vaccination program in Japan. Surveillance of the population-based incidence of CAP and serotype analysis of isolates causing CAP is fundamental to understand the impact of pneumococcal conjugate vaccine in pediatric CAP.

Cross-protection by the fusion pneumococcal surface protein A (PspA) vaccine against pneumonia caused by Streptococcus pneumoniae with five different PspA clades in mice

Kazunori Oishi[1)], Zhenyu Piao[2)], Yukihiro Akeda[2)], Kazunori Tomono[3)]

1) National Institute of Infectious Diseases
2) Research Institute for Microbial Diseases, Osaka University
3) Division of Infection Control and Prevention, Osaka University

Limitation of polysaccharide-based vaccines is the requirement of multiple serotypes formulation and the replacement of serotype of invasive pneumococcal disease strains after their introduction. To develop a cross-protective pneumococcal protein vaccine, three fusion pneumococcal surface protein A (PspA) proteins (A, B, and C), containing two clades belonging to the Families 1 and 2, were generated and examined for the cross-reaction and cross-protection against pneumococcal isolates from invasive diseases. Mice were immunized subcutaneously three times with one of fusion PspA proteins in combination with adjuvants (CpG oligonucleotide K3 plus aluminum hydroxide gel). Sera were collected from immunized mice and examined for the immunogenicity to the PspA clades 1-5 by ELISA and for the whole cell binding to five strains with different PspA clades 1-5 using flow cytometry. Of three fusion PspA proteins, PspA A or PspA C with adjuvants induced the high titers (> 8) of PspA-specific IgG to all of five different PspA clades, but PspA B with adjuvants induced the high titers of PspA-specific IgG only to PspA clades 1,2,4 and 5, but not to PspA clade 3. The frequencies of PspA-specific IgG binding of immune sera raised by PspA3+2 were higher than 70% to all of five strains with different clades 1-5, but those of immune sera raised by PspA A or B were less than 50% to a strain with PspA clades 3. Immunization with PspA C could render a significant protection of mice against pneumonia induced by pneumococcal strains with all of five PspA clades, while immunization with PspA A or PspA B protected mice against pneumonia induced by strains with clades 2,4 and 5 or a strain with clade 1, respectively. Our data demonstrated a cross-protection by immunization with PspA C in combination with adjuvants against pneumonia caused by pneumococcal strains with a wide range of PspA clades 1-5. A fusion PspA C protein can be a possible candidate of pneumococcal protein vaccines.

Invasive Pneumococcal Diseases in Bangladesh - Possible Impact of Pneumococcal Conjugate Vaccine

Samir K. Saha

Child Health Research Foundation, Department of Microbiology, Dhaka Shishu Hospital, Dhaka - 1207, Bangladesh

In 2006, the World Health Organization (WHO) recommended routine immunization with pneumococcal conjugate vaccines (PCV) for all countries. A recent meta-analysis showed that Invasive pneumococcal disease (IPD) is accounting for 11% of all deaths among children 1 - 59 months in the year 2000 worldwide.

Though Asia is lagging behind in generating data on the burden of Invasive Pneumococcal Disease (IPD), multiple investigators in Bangladesh have collected high quality pre-PCV implementation data on disease burden from population based field sites and high throughput sentinel sites, supported by GAVI's PneumoADIP and WHO. In addition to a high burden of IPD, the data of last several years clearly showed that Pneumococcal serotype distribution in Bangladesh is diverse with frequent changes over time. This leads to a relatively low and variable coverage of PCVs, which is oscillating around 45%.

The Bangladesh Government has taken a bold decision to introduce the PCV in 2013 in its routine immunization program considering the high burden of pneumococcal pneumonia and meningitis in the country. The next challenge for Bangladesh is to monitor the impact of this vaccine. This is particularly important considering the available disease burden data from Bangladesh and the varied post-PCV introduction experience in different countries. The Bangladesh government is very open to such an impact assessment and is eager to work with researchers to produce the evidence needed for sustained policy support for the PCV immunization, well beyond the period of the GAVI support.

The presentation will describe the burden of IPD in Bangladesh and the possible impact of PCV on these diseases. In addition, country plan to monitor the impact of PCV will also be illustrated.

Linking innate immune pathways to antibody responses of protein–polysaccharide conjugate vaccines: Comparison of meningococcal polysaccharide versus meningococcal conjugate vaccines in healthy adults

David S. Stephens, MD

Understanding how early innate immune responses influence the adaptive protective responses to vaccines is the focus of the new field of systems vaccinology. The goal is identify early innate signatures which correlate with and can predict subsequent differences vaccine immunogenicity which may impact vaccine efficacy or effectiveness such as the induction of mucosal immunity. Innate responses to the quadrivalent (A, C, Y, W-135) meningococcal polysaccharide vaccine (Menomune®,MPS) and meningococcal polysaccharide (DT) conjugate vaccine (Menactra®, MCV4) were evaluated in vivo. Healthy adults were vaccinated with MPS (n=13) or MCV4 (n=17). Gene expression microarrays of PBMCs obtained at days 0, 3 and 7 were evaluated. Serum bactericidal activity (SBA) to serogroups A and C *Neisseria meningitidis* was assessed in sera collected at days 0, 30, 180 and 730.

Both MPSV4 and MCV4 enhanced protective SBA titers which began to wane after 2 years. MCV4 and MPS highly upregulated genes such as TNFRSF17 (tumor necrosis factor receptor superfamily, member 17), shown to be a predictor of antibody responses for other vaccines. MCV4 induced a stronger transcriptomic response in day 7 that was highly enriched for B cell signatures. This was a recall plasmablast cell response, which was largely specific for the carrier (DT)protein and which was also reflected in a plasma cell, monocyte and TLR molecular signatures in PBMC transcriptomes at Day 3. This response program is shared by MCV4 (to DT) and Influenza TIV (HAI). A different polysaccharide-specific response program was seen for both MPSV4 and MCV4 with early activation (Day 3) of a dendritic cell and complement pathway molecular signatures in PBMC transcriptomes that correlated with the later (Day 30) anti-PS antibody responses. A booster study to evaluate polysaccharide memory responses is a next step

Both MPS and MCV4 induce significant increases in SBA titers against meningococcal serogroup A and C *N. meningitidis*. Meningococcal polysaccharide and conjugate vaccines provide a framework for evaluating similarities and differences in innate signatures generating specific antibody responses to these vaccines and to other antibody-inducing vaccines.

Etiology of Community Acquired Pneumonia in Adults Requiring Hospital Admission to a Tertiary Hospital in the Central Philippines

Socorro Lupisan[1)], Akira Suzuki[2)], Noel Macalalad[1)], Rutchie Egos[3)], Lydia Sombrero[1)], Michiko Okamoto[2)], Melisa Mondoy[1)], Hazel Galang[1)], Vicente Francisco Zeta[3)], Flora De la Pena[3)], Vicente Romano[1)], Remigio Olveda[1)], Hitoshi Oshitani[2)]

1) Research Institute for Tropical Medicine, Metro Manila, Philippines
2) Dept. of Virology, Tohoku University Graduate School of Medicine, Sendai, Japan
3) Eastern Visayas Regional Medical Center, Leyte, Philippines
4) Tohoku-RITM Research Center on Emerging and Reemerging Infectious Disease, Metro Manila, Philippines

Background:
Community acquired pneumonia (CAP) is the third leading cause of morbidity and mortality in the Philippines. There are very few local etiology studies in adults with CAP. The objective of this study is to determine the etiology and epidemiology of community-acquired pneumonia in the Eastern Visayas Regional Medical Center (EVRMC), Tacloban City, Leyte, the Philippines.

Methods:
This was a two-year prospective observational study. Patients > 14 years old with moderate or high risk CAP subsequently admitted at EVRMC, the biggest tertiary hospital in Central Philippines, from May 20, 2010 to May 30, 2012 were considered for enrolment to the study. Two nasopharyngeal swabs (NPS) were collected for viral studies. Sputum sampling was done for gram stain, aerobic culture, PCR for atypical bacteria and pertussis, AFB smear and TB culture. Blood was collected for culture/sensitivity testing. Empiric antibiotic treatment given on admission was based on the 2010 Philippine Clinical Practice Guidelines.

Results:
549 patients were enrolled; 90% with moderate risk pneumonia and 10% high risk pneumonia. Co-morbid condition in less than half (40%) was pulmonary disease and cardiovascular disease in 17.7%. More than half had diffuse infiltrates on chest X-ray and 25% had dense infiltrates. Influenza A (H3) (1.8%), RSV (2.2%), S. pneumoniae (3.5%) and M. tuberculosis (8.2%) were more common pathogens detected. The serotypes of S. pneumoniae detected were 5(n=5), 1(n=4), 2(n=3), 7(n=2), 12(n=2), and one each of 10, 25, and 7F. CFR was 13.7%, with the highest rate among high risk CAP patients (43.3%). Using univariate analysis, influenza A (H3), MRSA, E. coli were statistically significant causes of death.

Conclusion:
Viruses, bacteria and mycobacteria are important causes of CAP morbidity and mortality in the Philippines.

The relationship between biofilm formation and capsule in *Streptococcus pneumoniae* and *Haemophilus influenzae*

Hiroshi Watanabe and Liang Qin

Division of Infectious Diseases, Department of Infectious Medicine, Kurume University School of Medicine, Kurume, Japan

Background: *Streptococcus pneumoniae* and *Haemophilus influenzae* are the main cause of a variety of infections, including otitis media, meningitis and pneumonia. Recently, these organisms were reported to form biofilms, which is at the root of many persistent and chronic bacterial infections. The capsule is potentially related to virulence and biofilm formation. To address the relationship between biofilm formation and capsule, we conducted the following study.

Methods: Serotyping and PCR were performed to identify ß-lactamase-negative ampicillin-susceptible (BLNAS), ß-lactamase-negative ampicillin-resistant (BLNAR), TEM-1 type ß-lactamase-producing ampicillin-resistant (BLPAR)- Nontypeable *H. influenzae* (NTHi), and H. influenzae type b (Hib). We designed a capsular polysaccharide mutant, TIGR4cps4D–, from the TIGR4 *S. pneumoniae* strain. Biofilm formations were investigated by microtiter biofilm assay, as well as visually observation with a scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM) in a continuous flow chamber.

Results: The mean OD_{600} in the microtiter biofilm assay of 26 gBLNAS, 22 gBLNAR, 28 gBLPAR-NTHi, and 23 Hib strains were 0.57, 0.50, 0.34, and 0.08, respectively. NTHi strains were similar in terms of biofilm formations, which were observed by SEM and CLSM. Five Hib strains with the alternated type b cap genes showed significantly increased biofilm production than the other Hib strains. The mean OD_{595} in the microtiter biofilm assay of TIGR4cps4D– was 1.77 and 1.74, whereas that of TIGR4 was 0.76 and 0.33 on day 1 and day 2, respectively. SEM and CLSM showed TIGR4cps4D– formed a biofilm that was significantly thicker than that formed by TIGR4 (~12.22 vs. ~6.29 μm).

Conclusion: Our data indicates the capsule may inhibit biofilm formation in *S. pneumoniae* and *H. influenzae*.

Distributions of pneumococcal serotypes, PspA families and antimicrobial resistances among upper respiratory tract infections during pre-vaccine periods in Japan.

Muneki Hotomi[1)], Masamitsu Kono[1)], Masanobu Hiraoka[1)], Susan K. Hollingshead[2)], David E. Briles[2)], Noboru Yamanaka[1)]

1) Department of Otolaryngology-Head and Neck Surgery, Wakayama Medical University, Japan
2) Department of Microbiology, University of Birmingham at Alabama, USA

Background. Currently available pneumococcal vaccines are based on capsular polysaccharides. The 7-valent pneumococcal conjugate vaccine (7PCV) is highly efficacious at preventing bacteremic disease in children under 5 years of age. Promising results regarding the prevention of pneumonia and AOM, reducing nasopharyngeal carriage of vaccine serotypes, and elicitation of herd immunity against vaccine serotypes have also been reported for PCV. However, the protection is restricted to the limited number of the serotypes included in the vaccine. In recent years the protection afforded by the conjugate vaccine has begun to be eroded by an increasing frequency of infections with pneumococcal strains not covered by the vaccine. In the present study, we evaluated the distribution of the pneumococcal capsular type and surface protein A (PspA) family of pneumococcal isolates from upper respiratory tract infections in Japan.
Methods. A total of 251 *S. pneumoniae* isolates collected nationwide from patients seeking treatment for upper respiratory tract infections between January and May 2003 were used in this study. Their capsular tserotypes, PspA family and antibiotic resistance were characterized.
Results. Based on their susceptibility to PCG, the 251 pneumococcal isolates evaluated in this study were classified into three groups as follows: 93 (37.0%) PSSP, 104 (41.4%) PISP, and 54 (21.6%) PRSP. The most common serotype was 19F (20.7%) followed by 23F (16.3%), 6B (14.7%), 14 (8.0%), 6A (6.4%) and 3 (5.6%). Based on the macrolide susceptibilities, the 251 isolates were classified into four groups as follows: 106 (42.2%) strains with the *ermB* gene, 75 (29.8%) strains with the *mefA* gene, 15 (6.0%) strains with both genes, and 55 (22.0%) strains without both genes.

Among the 251 pneumococci isolates studied, the 49.4% were identified as belonging to family 2 (PspA2), and 44.6% to family 1 (PspA1). Thus, 94.0% of the isolates included in this study were PspA1- or PspA2-positive isolates. Eight isolates (3.2%) classified into PspA family 3 (PspA3). Four isolates (1.6%) were classified as PspA NT. Three isolates (1.2%) was identified as a PspA null strain.

The total serotype coverage of the 7-valent, 10-valent (10PCV), 13-valent (13PCV), and 23-valent pneumococcal vaccines were 62.2%, 62.9%, 76.9% and 70.5%, respectively. The coverage of DRSP by the 7-valent, 10-valent, 13-valent, and 23-valent pneumococcal vaccines were 74.7%, 75.3%, 82.9% and 78.5%, respectively.
Conclusion. The vast majority of pneumococci isolated from the middle ear fluids, nasal discharges/sinus aspirates or pharyngeal secretions represented PspA families 1 and 2. Capsular serotypes were generally not exclusively associated with certain PspA families, although some capsular types showed a much higher proportion of either PspA1 or PspA2. A PspA-containing vaccine would potentially provide high coverage against pneumococcal infectious diseases because it would be cross-protective versus invasive disease with the majority of pneumococci infecting children and adults. Even conjugate vaccine formulations with 13 pneumococcal capsular polysaccharides will not reach the coverage of 90 % or more achieved by a vaccine containing family 1 and 2 PspA. The addition of PspA to the existing conjugate vaccine formulations may be a possible alternative for future development of pneumococcal vaccine.

Microbiota of nasopharynx

Masanobu Hiraoka[1)], Muneki Hotomi[1)], Hatsumi Taniguchi[2)], Noboru Yamanaka[1)]

1) Department of Otolaryngology-Head and Neck Surgery, Wakayama Medical University
2) Department of Microbiology, University of Occupational and Environmental Health JAPAN

The upper respiratory tract infections such acute otitis media (AOM) and acute rhinosinusitis are popular among children worldwide. The most common pathogens like *Streptococcus pneumoniae* and *Haemophilus influenzae* are normal and transient residents of the nasopharyngeal niche, where they are embedded in a complex microbiota of generally presumed harmless commensals.

Nasopharyngeal bacterial colonization evolves rapidly during the first year of life. *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* colonize the nasopharynx early in life and are responsible for the vast majority of acute otitis media (AOM) and acute rhinosinusitis (ARS). Our previous study revealed the dramatic changes in the nasopharyngeal pneumococcal density during the course of upper respiratory infections.

In the nasopharynx, coexisting with causative pathogens and commensal consist bacterial flora, recentaly nominated as nasopharyneal microbiota. The microbiome in the nasopharynx will be dramatically changed during the course of antimicrobial treatments, vaccination and acute phase of upper respiratory inflammation. Despite an abundance of data on incidence, prevalence and density of potential pathogens in NP microbiota of children and adults, the detailed composition of the NP microbial community, both during health and disease have not been studied. We, therefore, will discuss microbita in the nasopharynx for better understanding the change of nasopharyngeal bacterial flora during various infectious situations.

Transmission dynamics of Human rhinovirus over a three-year period in pediatric cases of severe respiratory illness

Naoko Fuji[1)], Akira Suzuki[1,2)], Socorro Lupisan[3)], Lydia Sombero[3)], Taro Kamigaki[1,2)], Raita Tamaki[1,2)], Mariko Saito[1,2)], Rapunzel Aniceto[2,4)], Remigio Olveda[3)], Hitoshi Oshitani[1,2)]

1) Department of Virology, Tohoku University of graduated school of Medicine, Sendai Miyagi, Japan
2) Tohoku-RITM Collaborating Research Center for Emerging and Re emerging Infectious Disease, Muntinlupa, Metro Manila, Philippines
3) Research Institute for Tropical Medicine, Muntinlupa, Metro Manila, Philippines
4) Eastern Visayas Regional Medical Center, Leyte, Tacloban, Philippines

Human rhinovirus (HRV) C was recently identified using molecular technique as the third species of HRV. HRVs (A and B) infection is known to show higher circulation in fall and spring in template zone. However the seasonality in tropical area including novel HRVC are not fully studied yet.

Reconstructing transmission dynamism during May 2008 to May 2012 among hospitalized children with severe respiratory infections in the Philippines can provide knowledge on HRVs infection. We analyzed association of detection rate of intra and inter species, as well as serotypes. By combining serotypes, sequences and spatial data, we tried to show that the dynamism of HRVs circulation.

There were fluctuations of monthly detection rate. However those were not significantly associated to the spatial size of circulation. The spatial mean centres of monthly basis detected cases were moving around the location. And the dispersal patterns of case distributions were varied. Within a three year period, HRVA: 24, HRVB: 1, HRVC: 22 types were detected. We found that co-circulation of many serotypes. Some types appeared during several months, but some types were during limited periods. Furthermore there seems like no spatial and time association even within same serotypes. Patterns or mechanisms of co-circulation of variety of HRVs at limited geographic location were still unknown in this study.

Those data calls for the further study questions how the virus circulates within a community and sustains the transmission of many types at the same time. Community level studies are needed to overcome limitations which were especially this study's targeting population and the number of cases.

Molecular epidemiology and evolution of Enterovirus 68 in the Philippines, 2008-2011

Tadatsugu Imamura[1)], Akira Suzuki[1)], Socorro Lupisan[2)], Michiko Okamoto[1)], Raita Tamaki[1)], Mariko Saito[1)], Naoko Fuji[1)], Noel G. Macalalad[2)], Amado Tabdoc III[2)], Lydia Sombrero[2)], Remigio Olveda[2)], Hitoshi Oshitani[1)]

1) Tohoku University School of Medicine, Sendai City, Japan
2) Research Institute for Tropical Medicine, Muntinlupa City, the Philippines

Human enterovirus 68 (EV68) is a member of human enterovirus D (HEV-D), which belongs to Enterovirus species, Picornaviridae family. EV68 was first isolated in 1962 from 4 pediatric patients hospitalized with lower respiratory infection, in California. The number of reported EV68 cases has increased remarkably in recent years, and reported cases were associated with acute respiratory infections including a considerable number of severe cases.

In order to clarify the etiological and epidemiological significance of EV68 in the Philippines, we conducted a prospective study for EV68 in Leyte island, the Philippines, from May 2008 to December 2011, which include three studies; pediatric pneumonia study since May 2008, adult pneumonia study since May 2010 and influenza-like illness (ILI) study since January 2010. We identified EV68 in respiratory specimens collected from 9 pediatric pneumonia cases, 2 adult pneumonia cases, and 1 influenza-like illness case in the Philippines between June and August 2011. Combined with our previous report of EV68 outbreak in 2008-09, EV68 outbreaks in one area were identified twice with a 2-year interval. EV68 cases were most frequently detected among hospitalized children followed by hospitalized adults, both of which included one fatal case each. Our study suggests the clinical importance of EV68 as possible causative agent for severe respiratory infections.

Current Status of Mycoplasma pneumoniae infections in Japan

Tsuyoshi Kenri and Keigo Shibayama

Department of Bacteriology II, National Institute of Infectious Diseases, Japan

In Japan, mycoplasma pneumonia is classified as a category V infectious disease by the National Epidemiological Surveillance of Infectious Diseases under the Infectious Diseases Control Law. On the basis of this system, approximately 500 sentinel clinics in the country produce a weekly report of the number of mycoplasma pneumonia patients (inpatient and outpatient). The collected data are compiled by the National Institute of Infectious Diseases. The surveillance reports revealed a large increase in the number of infected patients in 2011 and 2012, which was 2–3 times higher than that observed during the other periods, since the surveillance commencement in 1999. Although the underlying cause for this increase remains unknown, several probable reasons have been pointed out. One is the periodic nature of mycoplasma pneumonia epidemics, which is a globally recognized phenomenon. Epidemiological studies from many countries reported that large epidemics of mycoplasma pneumonia occurred at 3–8-year intervals. This property was also previously observed in Japan between the 1970s and 1980s. Large epidemics of mycoplasma pneumonia occurred at 4-year intervals during this period. Although these periodical epidemics were not observed in Japan after the 1990s, there is a possibility that this periodicity within mycoplasma pneumonia epidemics has resurfaced. Another probable reason is the spread of macrolide-resistant Mycoplasma pneumonia. Macrolide-resistant clinical strains of M. pneumoniae were first isolated in Japan in 2000. Subsequently, the incidence of resistant strains gradually increased, especially in Asia. Currently, more than 50% of the clinical isolates from Japan are estimated to have resistant mutations. The increase in the incidence of macrolide resistance among these pathogens may affect the number of infected patients. In this study, we have analyzed approximately 50 clinical strains of M. pneumoniae isolated during the 2011–2012 epidemic period. Investigations of drug resistance and genotyping of these isolates have also been described.

Difference in the profile of locally produced cytokines/chemokines in pneumonia and encephalitis of mice

Masato Yashiro, Mutsuko Yamada, Yosuke Fujii, Yukie Saito, Hirokazu Tsukahara, Tsuneo Morishima

Departments of Pediatrics, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan

Introduction

Influenza virus infections cause widespread morbidity and mortality. Pandemic in 2009/2010 caused severe pneumonia and respiratory failure, and H5N1 may cause encephalitis in some cases, through virus replication in the brain. The current therapies for severe influenza complications are not satisfactory. In this study, we examined the difference in the profile of locally produced cytokines/chemokines in encephalitis and pneumonia of mice in order to establish effective treatment.

Methods

C57BL6J mice were infected with influenza virus A/WSN/33(H1N1). Pneumonia group (Group P) mice were inoculated intranasally, and encephalitis group (Group E) mice were inoculated intracranially (IC). Sera, bronchoalveorar lavage fluid (BALF), cerebrospinal fluid(CSF), lung tissues and brain tissues were collected from mice on Day 1, 3 and 5. Cytokines/chemokines were measured using multiplexed bead array system, and examined the magnitude and kinetics in the systemic site and the local site. The viral load in lung and brain tissues was measured using Real-time quantitative PCR. Pathological assessment of viral antigen, neutrophil and microglia was also performed.

Results

The viral antigen was detected by Day3, and neutrophil infiltration was confirmed in Group P. In Group E, the viral antigen was detected in the third ventricular, the fourth cerebral ventricle, and the cerebral cortex on Day3, and accumulation of microglia was confirmed in the third and fourth cerebral ventricle. In sera on Day3, inflammatory cytokines/chemokines such as IFNγ, IL-12, MCP-1, IP-10, and TNFα were induced in group P and E in a same manner. In the BALF of Group P, inflammatory cytokine/chemokines increased. In the CSF of Group E, interestingly, besides inflammatory cytokine, anti-inflammatory cytokines such as IL-10 and IL-13 increased.

Conclusion

In sera, regardless of the virus replication sites, cytokines/chemokines profile was almost same. In the local site (lung and brain), different cytokines/chemokines profile was found. Particularly, production of anti-inflammatory cytokines/chemokines was dominant in the CSF of mice with encephalitis. Understanding local immune response leads to establishment of effective treatment of severe influenza.

Ⅱ　分担研究報告

厚生労働科学研究費補助金（国際医学協力研究事業）
分担研究報告書

インフルエンザ感染における
局所サイトカイン・ケモカインの動態

研究分担者　森島恒雄　（岡山大学大学院小児医科学　教授）
研究協力者　八代将登　（岡山大学大学院小児医科学　助教）

研究要旨

肺炎および脳炎・脳症を引き起こす病原体が多数報告されているが、局所における免疫反応は明らかにされていない。インフルエンザウイルスの局所免疫応答を明らかにするために、マウスの肺および脳で増殖するA/WSN33(H1N1)を用いて実験を行った。

全身でのサイトカインの動態は血清を用いて検討した。肺炎群・脳炎群ともにG-CSF, IFNγ, IL-12, MCP-1, IP-10, TNFαなど、ほぼ同様の炎症性サイトカインが上昇していた。局所でのサイトカインの動態は、肺炎群では気管支肺胞洗浄液（BALF）を、脳炎群では脳脊髄液（CSF）を用いて検討した。肺炎群ではIP-10が、脳炎群では抗炎症性サイトカインであるIL-10, IL-13が有意に上昇していた。感染局所は病態を反映しやすいため、局所の免疫反応を評価することが重症インフルエンザの病態解析に重要であると考えられた。

A．研究目的

インフルエンザウイルスは、従来の季節型インフルエンザに加え、パンデミックを起こした2009年H1N1インフルエンザや、アジアを中心に拡がりをみせている強毒型のH5N1鳥インフルエンザなど、ヒトにおける重要な病原体の1つである。また、インフルエンザは小児や、糖尿病・気管支喘息などの基礎疾患を持つ患者を中心に重症化しやすく、急性呼吸窮迫症候群(ARDS)やインフルエンザ関連脳炎/脳症など重篤な病態を引き起こす。しかし、これらの病態における局所の免疫応答はまだ明確には解明されていない。本研究ではインフルエンザによる肺炎と脳炎における全身および局所でのサイトカイン・ケモカインプロファイルを比較検討した。

B．研究方法

マウス

8週齢雄C57BL/6マウス(Charles River Laboratories Japan Inc.)を用いた。このマウスは25℃に保たれた無菌室内で飼育された。