

The 2nd International Young Researcher Seminar in Zoonosis Control 2010

Programme & Abstract

HOKKAIDO UNIVERSITY HOKKAIDO UNIVERSITY HOKKAIDO UNIVERSITY HOKKAIDO UNIVERSITY

Duration September 13 (mon) -14 (tue), 2010
Venue Graduate School of Veterinary Medicine
Hokkaido University
Kita 18-jyo, Nishi 9-chome, Kita-ku, Sapporo, Japan

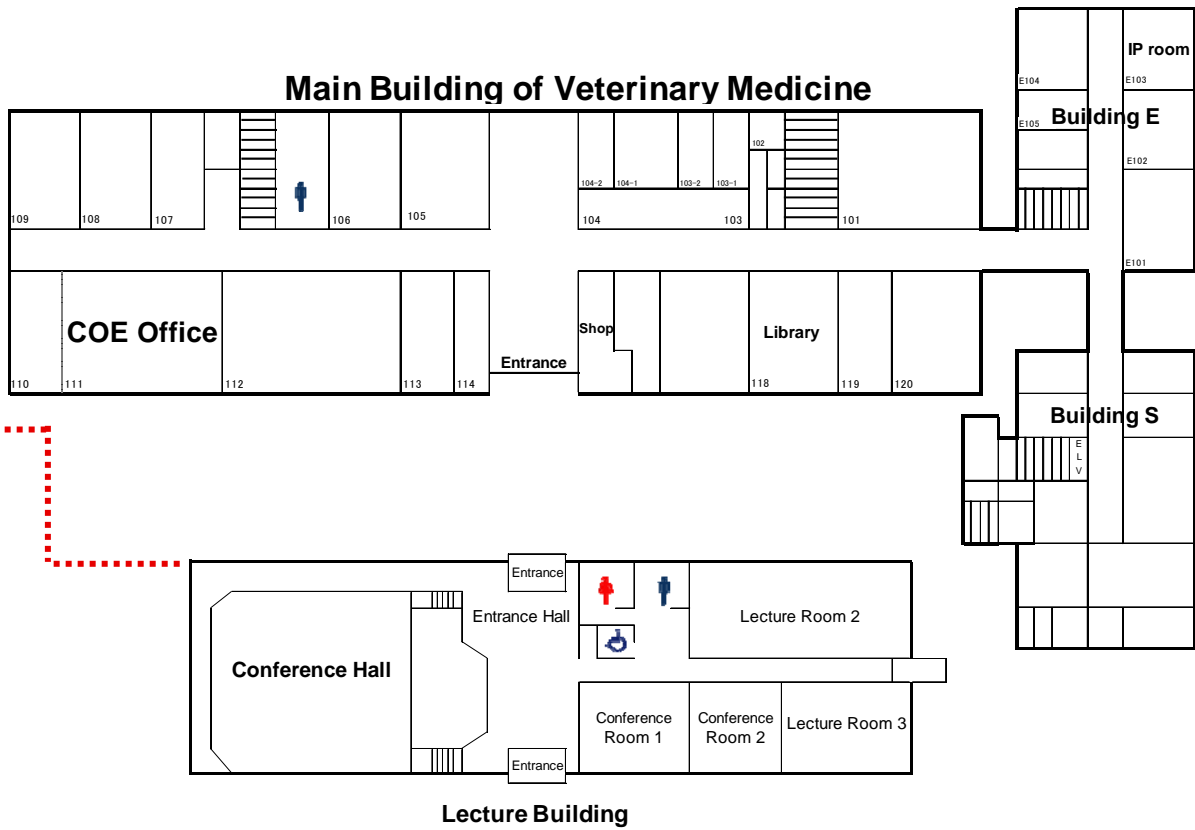
Organized by
Global COE Program, Hokkaido University
"Establishment of International Collaboration Centers for Zoonosis Control"

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Venue:





The seminar will be held at Lecture Building of Veterinary Medicine, Hokkaido University

Entrance Hall: Registration

Conference Hall: Presentation , Flash talk

Lecture room 2 & 3 : Poster session

Conference Room 1 & 2: Luncheon meeting and Welcome reception on Sept. 13

General Information & Guideline

General Information

Registration

You do not need submit any registration form, just tell your name at the desk of the lobby of Conference Hall, Veterinary Medicine from 8:30 to 9:30 on September 13 (Mon). You will have a programme & abstract, name card and information.

Registrationは9月13日(月)朝8時半から北大獣医学研究科講義棟 ロビーの受付にておこないます。受付でお名前を申し出てください。事前にRegistration formを提出する必要はありません。

Accommodation in Sapporo for the invited speakers

Sapporo Aspen Hotel

Address/ 5, Kita8-jyo Nishi4-chome, Kita-ku, Sapporo, Hokkaido 060-0808 Japan

Phone +81 11-700-2111

FAX +81 11-700-2002

<http://www.aspen-hotel.co.jp/english/frame.htm>

General Guideline

Oral presentation

- The laptops running Windows XP Professional operating system, with MS Office 2007 and Macintosh OS X operating system, with MS Office 2004 will be equipped.
- You can have your presentation in your own personal laptop if you use "moving images" or special programs included in your Power Point.
- Please keep the time for the presentation to ensure smooth proceedings.
- Please bring your presentation loaded in USB thumb drive (flash disk) or CD-ROM at the registration desk in the morning of Sept. 13.

【Keynote lectures】

Keynote presentation for Dr. Ali Mirazimi and Dr. Yoshizo Asano is allocated 60 min including discussion.

【10 Invited speakers (Oral presentation)】

Oral presentation for invited speakers is allocated 20 min including discussion.

【13 speakers from Hokkaido University】

Oral presentation for 13 speakers from Hokkaido University is allocated 15 min including discussion.

Note: All participants (including invited and oral presenters from Hokkaido University) should prepare poster.

However, invited and oral presenters from Hokkaido University do not need prepare "One minute presentation (flash talk)".

General Guideline for Poster Presentation

- Each board size is 900 mm W x 2,000 mm H
- A0 size: 841 mm x 1189 mm may be appropriate if you prepare your poster as a single sheet.
- Boards with Poster No. card and pushpins for poster setup will be provided.
- Each poster will be provided a poster number as indicated in the Programme & Abstract booklet.
- Poster is to be mounted from 8:30 to 9:30 in the morning of Sept. 13.
- Poster is to be removed from 18:00 to 18:30 on September 14.

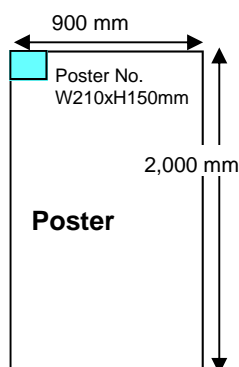
One minute presentation (flash talk) of 37 posters from Hokkaido University

Flash talk will be held from 16:15 to 17:00 on September 13.

The presentation should be one minute and prepare one ppt slide by September 8 and send it to GCOE office.

【One minute presentation (Flash talk) の進め方】

- 1) 一人あたりの持ち時間は70秒とする(約60秒発表)。
- 2) 全ての発表者は次演者待機場所に発表順に一列に並んでください。
- 3) 座長は名前と所属だけを紹介し、呼ばれたら1名ずつ壇上にご上がり、スライド1枚、約60秒間で発表を行う。
(残りの10秒は発表者の移動と座長による発表者紹介の時間とする。また、この場で討論の時間はない。)



Organizing Committee

Kentaro Yoshii

(Lab. of Public Health, Dept. of Environmental Veterinary Sciences, Graduate School of Veterinary Medicine, Hokkaido University, e-mail: kyoshii@vetmed.hokudai.ac.jp)

Satoru Konnai

(Lab. of Infectious Diseases, Dept. of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, e-mail: konnai@vetmed.hokudai.ac.jp)

Yuji Sunden

(Lab. of Comparative Pathology, Dept. of Veterinary Clinical Sciences, Graduate School of Veterinary Medicine, Hokkaido University, e-mail: sunden@vetmed.hokudai.ac.jp)

Masatoshi Okamatsu

(Lab. of Microbiology, Dept. of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, e-mail: okamatsu@vetmed.hokudai.ac.jp)

Tatsuya Sakurai

(Lab. of Parasitology, Dept. of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, e-mail: tsakurai@vetmed.hokudai.ac.jp)

Rie Hasebe

(Lab. of Veterinary Hygiene, Graduate School of Veterinary Medicine, Hokkaido University, e-mail: r-hasebe@vetmed.hokudai.ac.jp)

Shiro Murata

(Lab. of Infectious Diseases, Dept. of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, e-mail: murata@vetmed.hokudai.ac.jp)

Organized by

Global COE Program, Hokkaido University

“Establishment of International Collaboration Centers for Zoonosis Control”

GCOE Office

Graduate School of Veterinary Medicine 1F Room No. 111

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gcoe@vetmed.hokudai.ac.jp

<http://www.vetmed.hokudai.ac.jp/gcoe/>

FYI

Sapporo city

<http://www.welcome.city.sapporo.jp/english/index.html>

Narita International Airport (Tokyo)

<http://www.narita-airport.jp/en/index.html>

New Chitose Airport (Sapporo)

<http://new-chitose-airport.jp/en/>

JR Sapporo Station map

<http://www2.jrhokkaido.co.jp/global/english/access/sapporo.html>

Hokkaido University Campus

<http://www.hokudai.ac.jp/en/pickup/accesstocampus.html>

Sapporo Beer Garden

<http://www.sapporo-bier-garten.jp/foreign/english.php>

Program Day 1

September 13 (mon), 2010

8:30 ~ 9:30	Registration, Submitting presentation data and setting up poster
9:30 ~ 9:45	Orientation (Kentaro Yoshii, Organizing Committee) Opening speech (Hiroshi Kida, Leader of Global COE Program, Professor, Hokkaido Univ.)
9:45 ~ 10:40	Oral presentation I (Chair persons: Satoshi Miyazaki & Hirohisa Mekata) O-1 Interactions between mutualist <i>Wigglesworthia</i> and tsetse peptidoglycan influence trypanosome transmission recognition protein (PGRP-LB) Jingwen Wang (Yale Univ., USA) O-2 Tsetse EP protein and its role in refractoriness to parasite infection Lee Haines (Liverpool School of Tropical Medicine, England) O-3 Establishment of an in vitro transgene expression system in epimastigotes of <i>Trypanosoma congolense</i> Tatsuya Sakurai (Hokkaido Univ.)
10:45 ~ 11:00	++ Break ++
11:00 ~ 11:50	Oral presentation II (Chair persons: Kyoko Hayashida & Junko Doi) O-4 Molecular biological tools for the immunization against <i>Theileria parva</i> Michiel E. Janssens (The Institute for Tropical Medicine, Belgium) O-5 Gene silencing in <i>Echinococcus multilocularis</i> protoscoleces using RNA interference Chiaki Mizukami (Hokkaido Univ.) O-6 Comparison of vaccine efficacy of tetraspanin3 (TSP3) protein by subcutaneous and intranasal immunization Zhisheng Dang (Hokkaido Univ.)
12:00 ~ 13:30	Luncheon meeting at Conference room (only for Invited speakers, GCOE members and Organizing committee)
13:30 ~ 14:30	Keynote Lecture I (Chair persons: Satoru Konnai & Shiro Murata) Control of varicella, one of the popular viral diseases in childhood, by the Oka vaccine Yoshizo Asano (Hokkaido Univ.)
14:30 ~ 14:45	++ Break ++
14:45 ~ 16:10	Oral presentation III (Chair persons: Yasuko Orba & Takaaki Koma) O-7 The ambivalent role of type-I-interferon in rabies virus immunosubversion and pathogenesis Damien Chopy (Institut Pasteur, France) O-8 Rabies Control Activities in Central Province, Sri Lanka Koji Kanda (Hokkaido Univ.) O-9 Alterations in membrane architecture in Flavivirus-infected cells Rushika Perera (Purdue Univ. USA) O-10 Chondroitin sulfate-E enhances Japanese encephalitis virus infection in neuronal cells Eunmi Kim (Hokkaido Univ.) O-11 Bovine and Murine Macrophage degradation of scrapie and BSE PrP ^{Sc} Yukiko Sassa (Hokkaido Univ.)
16:15 ~ 17:00	Flash Talk
17:00 ~ 18:00	Poster core time I (Odd numbers and oral presentations at day 1) at Lecture room 2 & 3
18:00 ~ 19:30	Welcome reception at Conference room

Program Day 2

September 14 (Tue), 2010

9:15 ~ 10:25 **Oral presentation I V** (Chair persons: Shumpei P. Yasuda & Akira Kawaguchi)

- O-12 Cell entry of Lassa virus
Giulia Pasqual (Univ. of Lausanne, Switzerland)
- O-13 Hantaviruses and Arenaviruses coexistence in different geographic regions of Argentina
Carina Sen (National Institute of Human Viral Diseases, Argentina)
- O-14 Hantavirus nucleocapsid protein promotes cis-Golgi targeting of glycoprotein Gc
Kenta Shimizu (Hokkaido Univ.)
- O-15 Persistent infection of Puumala virus in Syrian hamsters (*Mesocricetus auratus*) resembling hantavirus infection in natural hosts
Takahiro Sanada (Hokkaido Univ.)

10:30 ~ 10:45 ++ Break ++

10:45 ~ 11:45 **Keynote Lecture II** (Chair persons: Kentaro Yoshii & Yuji Sunden)

- The molecular pathogenesis of Crimean Congo Hemorrhagic Fever Virus
Ali Mirazimi (Swedish Institute for Infectious Disease Control, Sweden)

12:00 ~ 13:30 Lunch

13:30 ~ 14:55 **Oral presentation V** (Chair persons: Naoki Yamamoto & Mami Matsuda)

- O-16 Insights into the 3D structure and assembly of influenza virus by cryo-electron microscopy and hybrid methods: New views of an old virus
Audray K. Harris (National Institutes of Health, Bethesda, USA)
- O-17 Recognition of the influenza virus hemagglutinin by neutralizing antibodies
Damian C. Ekiert (The Scripps Research Institute, USA)
- O-18 Potency of the A/2009 (H1N1) pandemic influenza vaccine prepared from an isolate of swine origin, A/swine/Hokkaido/2/1981
Masatoshi Okamoto (Hokkaido Univ.)
- O-19 Protective Effect of HLA-A*2402 restricted CTL-inducing peptides against Influenza A virus infection
Toru Ichihashi (Hokkaido Univ.)
- O-20 Control mechanism for the infiltration of inflammatory cells after influenza A virus infection
Yosuke Nakayama (Hokkaido Univ.)

15:00 ~ 15:15 ++ Break ++

15:15 ~ 16:05 **Oral presentation VI** (Chair persons: Masayoshi Isezaki & Naoki Nomura)

- O-21 Disinfectant-resistant mycobacteria
Mary Jackson (Colorado State University, USA)
- O-22 Epidemiological Study of Tuberculosis in Lechwe (*Kobus lechwe*) in Zambia and Anthroponosis between Human and Chimpanzee in Tanzania
Takanori Kooriyama (Hokkaido Univ.)
- O-23 Whole-genome analysis of an attenuated Ehrlichia ruminantium vaccine strain using next-generation sequencer
Ryo Nakao (Hokkaido Univ.)

16:15 ~ 17:15 Poster core time II (Even numbers and oral presentations at day 2) at Lecture room 2 & 3

17:30 ~ Award & Closing Speech (Chihiro Sugimoto, Professor, Hokkaido Univ.)

Un- ~ Sapporo Beer Garden
fixed

Profile of Invited speakers

Keynote lecture I

Yoshizo Asano

Professor (Specially Appointed) for Zambia Project
Research Center for Zoonosis Control
Hokkaido University
Japan
yasano@czc.hokudai.ac.jp



ACADEMIC DEGREES:

M.D. 1969 Nagoya University (Medicine)
Ph.D. 1978 Nagaya University (Medical Science)

PROFESSIONAL APPOINTMENTS:

1969-70 Physician, Department of Surgery, Meitetsu Hospital, Nagoya, Japan
1970-72 Pediatrician, Department of Pediatrics, Toyota Hospital, Toyota, Aichi, Japan
1972-73 Pediatrician, Department of Pediatrics, Nagoya University School of
Medicine, Nagoya, Japan
1973-77 Pediatrician, Department of Pediatrics, Chukyo Hospital, Nagoya, Japan
1977-79 Assistant Professor, Department of Virology, Research Institute for Microbial
Diseases, Osaka University, Suita, Osaka, Japan
1979-94 Associate Professor, Department of Pediatrics, Fujita Health University School
of Medicine, Toyoake, Aichi 470-11 Japan
1980-82 Visiting Scientist, Division of Virology, Bureau of Biologics, Food and Drug
Administration, Bethesda, MD
(Directors: Drs. FA Ennis & Paul Albrecht)
1985-86 Visiting Scientist, Division of Virology, Center for Drugs and Biologics,
Office of Biologics Research and Review, Food and Drug Administration,
Bethesda, MD
(Director: Dr. Gerald V. Quinnan, Jr.)
1994-2010 Professor and Chair, Department of Pediatrics, Fujita Health University School
of Medicine, Toyoake, Aichi 470-11 Japan
2010- Professor (Specially Appointed) for Zambia Project, Research Center for
Zoonosis Control, Hokkaido University

RESEARCH INTERESTS:

Pathogenesis, diagnosis, prevention, and treatment of viral infections (mainly varicella-zoster virus, human herpesvirus 6, and human herpesvirus 7) in childhood.

Ali Mirazimi

Associate Professor
Head of Highly pathogenic virus section
Centre for Microbiological preparedness
Swedish Institute for Infectious Disease Control and
Institute for Microbiology, Tumor Biology and cell biology
Karolinska Institute
Sweden
Ali.mirazimi@smi.se



ACADEMIC DEGREES:

B.A. 1994 Karolinska Institute (Biomedical science)
Ph.D. 2000 Karolinska Institute (Infection Biology)

PROFESSIONAL APPOINTMENTS:

2001-2002 Lecturer, Karolinska Institute
2002-2004 Project leader, Swedish Institute for Infectious Disease Control
2006-present Associate Professor, Karolinska Institute
2004-2010 Head of Section, Institute for Infectious Disease Control
2009-present Deputy Head of dept, Institute for Infectious Disease Control

RESEARCH INTERESTS:

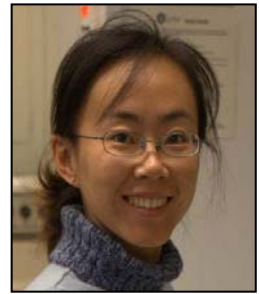
Highly pathogenic viral Zoonosis with focus to study the virus-host cell interaction, and understand the molecular pathogenesis, developing diagnosis for emerging viral diseases and also understand the evolution and migration of viral zoonosis around the world.

Profile of Invited speakers

Oral presentation 1

Jingwen Wang

Postdoc Associate
Department of Microbial Disease
School of Epidemiology and Public Health
Yale University, U.S.A.
jingwen.wang@yale.edu



ACADEMIC DEGREES:

B.A. 2001 Shanghai University, China
Ph.D. 2006 Fudan University, China
Post-Graduate Student 2006 Yale University

PROFESSIONAL APPOINTMENTS:

2006- Postdoctoral Associate, Yale University

RESEARCH INTERESTS:

We are interested in understanding multiple aspects of tsetse immune system related to symbiosis and trypanosome infection. Our main focus is to investigate how tsetse immune system recognize self and non-self and how they react differentially to these microbes, allowing symbionts to proliferate, while limit parasites growing. Currently people in our lab have characterized several molecules of tsetse Toll and Imd pathway which play important roles in both symbionts controlling and parasites defending. To further understand interplay between them, we will compare sequences between laboratory reared colony and field flies, refractory and sensitive species by parallel deep sequencing. In addition, we are able to maintain symbionts free flies by artificially feeding flies with antibiotics. These flies will be good models for us to study the effect of symbionts on tsetse immune system development and on trypanosomes prevention. By studying the relationship among tsetse, its symbionts and trypanosome, it will help us develop solid approaches to control African sleeping sickness.

Oral presentation 2

Lee Haines

Post Doctoral Researcher
Vector Research Group
Liverpool School of Tropical Medicine
Liverpool UK
lhaines@liv.ac.uk



ACADEMIC DEGREES:

B. Sc. 1994 Trinity Western University (Biology)
M. Sc. 2002 University of Victoria (Biochemistry and Molecular Biology)
Ph.D. 2009 Liverpool School of Tropical Medicine (Tropical Medicine)

PROFESSIONAL APPOINTMENTS:

1995-99 Organ Transplant and Tissue Typing Division Manager,
Cedarlane Laboratories, Mississauga, Ontario, Canada
2002- 05 Lab Manager of Molecular Parasitology Group, University of Victoria
Victoria, British Columbia, Canada
2010 Post Doc, Vector Biology, Liverpool School of Tropical Medicine
Liverpool, England

RESEARCH INTERESTS:

Host-parasite interactions, midgut ecology, symbiosis, proteomics, vector biology, molecular entomology, vaccine development, parasitology, host immunity.

Oral presentation 4

Michiel Janssens

Post-doctoral collaborator
Division of Animal health
Unit of Veterinary Parasitology
Institute of Tropical Medicine Antwerp
Belgium
mjanssens@itg.be



ACADEMIC DEGREES:

B.A. 2002 University of Antwerp (Biology)
M.A. 2004 University of Antwerp (Biology)
Ph.D. 2009 University of Antwerp (Science)

PROFESSIONAL APPOINTMENTS:

2008-Present Post-doctoral collaborator at the Institute of Tropical Medicine Antwerp

RESEARCH INTERESTS:

Molecular Biology
Immunology
Parasitology

Oral presentation 7

Damien Chopy

PhD student,
Virology Departement
Neuro-Immunologie Virale Unit
Institut Pasteur, Paris, France
Pierre et Marie Curie University (Paris 6), France
dchopy@pasteur.fr



ACADEMIC DEGREES:

2006 Bachelor's Degree specialized in Biology Cellular and Molecular at Ecole Normale Supérieure of Lyon, passed with honors.

2008 Master's Degree in Sciences Cellular and Molecular Biology at Ecole Normale Supérieure of Lyon, France, passed with honors.

PROFESSIONAL APPOINTMENTS:

May to Aug. 2006 Training course in INSERM 758 Unit at Ecole Normale Supérieure head by Dr. François Loïc Cosset in Lyon, France. Analysis of the ability of fusogenic Associated Small Transmembrane (FAST) proteins to produce infectious retroviruses for gene therapy.

Feb. to Jul. 2007 Training period at John Hopkins school of public health in Dr Griffin laboratory, Baltimore, MD, USA. In vitro and in vivo analysis of the involvement of transcription factor Nrf2 and the cytokine IL-10 during infection of the alphavirus Sindbis virus.

Sept. to Dec. 2007 Training course in Virologie et Pathogénèse Virale unit CNRS FRE3011 managed by Dr Gerlier, Lyon, France. Development of an RNA interference strategy to study Measles virus polymerase.

Feb. 2008 to date : Internship in Neuro-Immunologie Virale Unit head by Dr. Lafon at Institut Pasteur, Paris, France.

Profile of Invited speakers

RESEARCH INTERESTS:

I am interested in the immune response during virus infection of the nervous system. I am focusing on the balance between the immune response and the neuronal homeostasis that allows rabies virus migration in the nervous system. I am developing physiopathology study in mouse model based on cellular and molecular approaches to better understand the host-rabies virus relationship. From this knowledge, we might be able to break the host-virus balance that leads to pathogenesis.

Oral presentation 9

Rushika Perera, Ph.D



Assistant Research Scientist
Markey Center for Structural Biology
Dept. of Biological Sciences
Purdue University, U.S.A.
perera@purdue.edu

ACADEMIC DEGREES:

B.A.	1995	Chemistry, Goshen College, Goshen, Indiana
B.A.	1995	Biology, Goshen College, Goshen, Indiana
Ph.D.	2002	Biological Sciences, Purdue University, West Lafayette, Indiana,

PROFESSIONAL APPOINTMENTS:

12/2005-Present	Assistant Research Scientist Markey Center for Structural Biology Department of Biological Sciences Purdue University, West Lafayette, IN
2002-2005	Post Doctoral Fellow Department of Microbiology and Molecular Genetics University of California, Irvine, CA

RESEARCH INTERESTS:

My research currently focuses on the flaviviruses. I use a multidisciplinary approach including Molecular and Cellular Biology, Biochemistry, and Structural Biology to study virus structure, replication, and pathogenesis to better understand how these viruses control their life cycle, interact with the host, and cause infectious disease.

I'm primarily interested in flavivirus-host interactions and the requirement of lipid biosynthetic pathways to support productive viral replication. Lipids have gained increased attention as primary mediators of human metabolic diseases and their contribution to the homeostasis of the intracellular environment is an attractive target for intracellular pathogens. Enveloped viruses are dependant on host lipid biosynthetic pathways to regulate the processes of virus entry, genome replication, packaging, assembly and release. Interestingly, this dependency translates across species from the human host to the mosquito vector. Unlike bacterial pathogens, the inability of these viruses to produce their own lipids in part drives their obligate parasitism of the host cell.

Oral presentation 12

Giulia Pasqual

PhD student
Institute of Microbiology
University Hospital Center and University of Lausanne
Switzerland
Giulia.Pasqual@chuv.ch



ACADEMIC DEGREES:

B.A. 2005 University of Padua, Italy (Biotechnology)
M.A. 2007 University of Padua, Italy (Medical Biotechnology)
Ph.D. Since 2008 University of Lausanne (Life Sciences)

RESEARCH INTERESTS:

Since the beginning of my PhD I am working, under the supervision of Prof. Stefan Kunz, on the interaction of arenaviruses with the host cell. In particular, the main aspect of my research is the cellular entry mechanism and intracellular trafficking of Lymphocytic choriomeningitis virus and Lassa virus.

Oral presentation 13

Carina Noe Sen

Biotechnologist
Virology Laboratory
Research Department
National Institute of Human Viral Diseases “Dr. Julio I. Maiztegui”,
Pergamino, Argentina
cari_sen@yahoo.com.ar



ACADEMIC DEGREES:

Biotechnologist, 2008
Faculty of Biochemical and Pharmaceutical Sciences. National University of Rosario. Santa Fe, Argentina

RESEARCH INTERESTS:

My primary research interest is the study of hantavirus and arenavirus infections associated with American rodents, with emphasis on the molecular and phylogenetic analysis of these groups of viruses.

Profile of Invited speakers

Oral presentation 16

Audray Harris

Research Fellow
Laboratory of Cell Biology
National Cancer Institute
National Institutes of Health, U.S.A.
harrisau@mail.nih.gov



ACADEMIC DEGREES:

B.S. 1995 Tougaloo College (Chemistry)
Ph.D. 2002 University of Alabama at Birmingham (Microbiology)

PROFESSIONAL APPOINTMENTS:

2003 - 2007 Post-doctoral fellow, National Institutes of Health
2008 - Research Fellow, National Institutes of Health

RESEARCH INTERESTS:

My broad research interest is to study the structure and assembly of viruses and to use this structural information to help design antiviral and vaccines. In addition, the use of structural information from different structural techniques such as x-ray and electron microscopy is at the core of my research methodology. One of the fundamental issues in virology is to understand the structure and assembly of virus particles and to use this structural information to facilitate the discovery of antivirals and vaccines. I have concentrated my efforts on three viruses: Hepatitis B, Influenza, and HIV. A particular interest is the structure and assembly of the influenza virus.

Oral presentation 17

Damian Charles Ekiert

PhD Candidate
Department of Molecular Biology and
The Skaggs Institute for Chemical Biology
The Scripps Research Institute
La Jolla, CA, USA
dcekiert@scripps.edu



ACADEMIC DEGREES:

A.B. 2005 University of Chicago (Biology)

RESEARCH INTERESTS:

Influenza viruses pose a continual threat to global public health. Vaccination remains the most effective control measure, but current vaccines elicit a predominantly strain-specific response and depend on a good match between the vaccine formulation and circulating viruses. When a novel strain arises unexpectedly, as in the case of a pandemic, current vaccines provide little protection. Using X-ray crystallography, we are working to understand how several recently-discovered, broadly neutralizing antibodies recognize the influenza virus hemagglutinin and block viral replication. The crystal structure of the first of these antibodies, CR6261, in complex with hemagglutinin revealed the presence of a highly conserved epitope in the stem region, distant from the sites recognized by other structurally characterized antibodies. CR6261 and related antibodies are in development as monoclonal antibody therapeutics for severe influenza infections and new vaccination strategies are being explored in order to efficiently elicit CR6261-like antibodies.

Mary Jackson

Assistant Professor
Department of Microbiology, Immunology and Pathology
College of Veterinary Medicine and Biomedical Sciences
Colorado State University, Fort Collins, U.S.A.
Mary.Jackson@ColoState.EDU



ACADEMIC DEGREES:

M.S. 1994 Ecole Nationale Supérieure Agronomique de Rennes / Institut Pasteur,
Paris, France (Biology and Agronomy)
Ph.D. 1998 Ecole Nationale Supérieure Agronomique de Rennes / Institut Pasteur,
Paris, France (Biochemistry, Molecular and Cellular Biology)

PROFESSIONAL APPOINTMENTS:

May 2007 – present: Assistant Professor in Microbial Pathogenesis, Dept. of Microbiology, Immunology and Pathology,
Colorado State University, Fort Collins, CO, USA
2002 - 2007: Assistant Professor in Mycobacterial Genetics, Unité de Génétique Mycobactérienne, Institut Pasteur,
Paris, France
2000 - 2001: Research Scientist, Unité de Génétique Mycobactérienne, Institut Pasteur, Paris, France.

RESEARCH INTERESTS:

Mycobacterial genetics and biochemistry
Molecular aspects of mycobacterial diseases and biocide resistance
Development of new therapeutic strategies against *Mycobacterium tuberculosis*

Abstract Keynote Lecture

Keynote Lecture I (Day 1: 13:30-14:30 on September 13)

Keynote lecture I

Control of varicella, one of the popular viral diseases in childhood, by the Oka vaccine

Yoshizo Asano

Zambia Project,

Research Center for Zoonosis Control, Hokkaido University
yasano@czc.hokudai.ac.jp

Varicella is a highly contagious disease such as measles, mumps, and rubella in childhood. The disease is caused by the primary infection with varicella-zoster virus (VZV), and is generally believed to be benign except occasional complications in immunocompetent individuals, however, sometimes to be severe or fatal in immunocompromised hosts. More than 30 years ago we had no effective tools for controlling primary VZV infection, such as vaccine or acyclovir.

A live attenuated varicella vaccine was originally developed in Japan in 1974. The virus used was isolated from an otherwise healthy 3-year-old Japanese boy (his family name is Oka) and was then attenuated by passaging through human embryonic lung cells, guinea pig embryonic cells at a low temperature. The vaccine is safe, highly immunogenic, and effective against varicella. It is now commercially available worldwide and is applied to approximately 16 million individuals of approximately 80 countries in 2006. The most dramatic changes were reported in the USA after introduction of universal immunization strategy in 1996. They include significant decline in varicella disease, varicella-related mortality, varicella-related hospitalizations and expenditures, and varicella-related complications. After the immunization, breakthrough cases of varicella were occasionally reported, however, clinical features of the breakthrough varicella were uniformly mild and less infectious than natural disease.

The Oka vaccine induces VZV specific T cell mediated immunity and reduced the burden of illness due to herpes zoster, the secondary infection due to reactivation of latent VZV in sensory ganglia. The vaccine is now applied to elderly individuals for prevention of herpes zoster.

Thus the Oka vaccine is now used for controlling primary infection of VZV and also reactivation of the latent virus.

Keynote Lecture II (Day 2: 10:45-11:45 on September 14)

Keynote lecture II

The molecular pathogenesis of Crimean Congo Hemorrhagic Fever Virus

Ali Mirazimi

Centre for Microbiological Preparedness
Swedish Institute for Infectious Disease Control, Sweden
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Crimean-Congo hemorrhagic fever virus (CCHFV) is the etiological agent of a human disease characterized by fever, prostration, severe hemorrhages and death. The first documented outbreaks were recorded in 1944 and 1945. Nowadays, CCHFV is known to be widely distributed throughout large areas (the virus is prevalent in about 30 countries in Asia, Africa and Europe). CCHFV is the second most widespread arbovirus of medical importance after Dengue virus. CCHFV also infects animals, but these remain asymptomatic as shown for cattle, sheep, goats, camels and hares. The virus can be transmitted to humans through ticks of the genus *Hyalomma*. Human infection also occurs by contact with blood or tissue material from infected animals or humans. In addition, person-to-person transmission can occur via bloody vomit, body fluids or by aerosol from patients in advanced stages of disease.

Clinical symptoms of infection commonly include hemorrhage, myalgia, and fever. Levels of liver enzymes are raised, and bleeding markers are often increased. Here, we will present the molecular mechanism behind the pathogenesis of this virus infection in vitro and in vivo. We have demonstrated that CCHFv employs a range of passive and active mechanisms to avoid induction of the antiviral type I interferons. The most recent findings demonstrate that CCHFv interact with different cellular pathway mechanism which can have impact in developing the pathogenesis in CCHFv infection.

Abstract Oral presentation Day 1: September 13

Oral presentation I
(Day 1: 9:45-10:40 on September 13)

O-1

Interactions between mutualist *Wigglesworthia* and tsetse peptidoglycan influence trypanosome transmission recognition protein (PGRP-LB)

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Tsetse flies, the sole vectors of African trypanosomes, rely on mutualistic endosymbionts *Wigglesworthia glossinidia* for fecundity. Elimination of *Wigglesworthia* render flies not only sterile, but also highly susceptible to trypanosome infections. We show that a tsetse peptidoglycan recognition protein (PGRP-LB) plays a critical role in symbiotic tolerance and trypanosome transmission processes. Our findings suggest that expression and regulation of *pgrp-lb* in tsetse differ from the closely related *Drosophila* as a result of tsetse's limited exposure to foreign microbes and symbiotic adaptations. PGRP-LB is expressed in *Wigglesworthia* harboring organ in the midgut and its level of expression increases in direct correlation with symbiont numbers. RNA interference (RNAi)-mediated depletion of *pgrp-lb* results in activation of the immune deficiency (IMD) signaling pathway and leads to the synthesis of antimicrobial peptides (AMPs), which decrease *Wigglesworthia* density and increase parasite infection prevalence. *Wigglesworthia*-free adults and parasitized flies have significantly less *pgrp-lb* levels than corresponding normal adults and resistant flies that successfully eliminated trypanosome infections, respectively. Based on the presence of conserved amidase domains, tsetse PGRP-LB likely scavenges peptidoglycan (PGN) released by *Wigglesworthia* to prevent activation of symbiont damaging host immune responses. Tsetse PGRP-LB also apparently has a trypanosome inhibitory function as its absence increases host parasite susceptibility, and leads to a high parasite infection prevalence. A dynamic interplay between *Wigglesworthia* and host immunity apparently modulates tsetse's susceptibility to trypanosomes and influences symbiont viability and host fecundity outcomes.

Oral presentation I
(Day 1: 9:45-10:40 on September 13)

O-2

Tsetse EP protein and its role in refractoriness to parasite infection

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African trypanosomes undergo a complex developmental cycle in their tsetse fly vector before transmission back into a vertebrate host. Typically 90% of fly infections fail, most during initial establishment of the parasites in the fly midgut. The specific mechanism(s) underpinning this innate refractoriness are unknown. We have previously reported that the tsetse fly, in response to gram-negative microbial challenge, up regulates a *Glossina*-specific, immunoresponsive molecule called the tsetse EP protein. Here we show, by RNA interference, that this tsetse EP protein acts as an antagonist of trypanosome establishment in the fly midgut. We demonstrate that this phenomenon exists in two species of tsetse, *Glossina morsitans morsitans* and *Glossina palpalis palpalis*, suggesting that tsetse EP protein may be a conserved determinant of vector competence in all *Glossina* species. The knockdown of tsetse EP protein also resulted in a significant increase in the prevalence of midgut parasite infections with both *T. b. brucei* and *T. congolense*. This elevated susceptibility to trypanosome infection following gene knockdown was witnessed with all ages of tsetse investigated. Tsetse EP protein levels naturally decline in response to starvation, suggesting the increased susceptibility to trypanosome infection of starved flies may be linked to tsetse EP protein levels. As starvation is a common field event, this fact may be of considerable importance in the epidemiology of African trypanosomiasis.

Oral presentation I (Day 1: 9:45-10:40 on September 13)

O-3

Establishment of an *in vitro* transgene expression system in epimastigotes of *Trypanosoma congolense*

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Trypanosoma congolense epimastigote forms (EMFs) adhere to the tsetse fly proboscis, proliferate, and differentiate into animal-infective metacyclic forms (MCFs). This differentiation step, called metacyclogenesis, is indispensable for the cyclical transmission of the parasite. Although an *in vitro* metacyclogenesis culture system was established several decades ago, few genetic tools have been utilized to investigate the molecular mechanisms underlying *T. congolense* metacyclogenesis. This study established a transgene expression system using an *in vitro* derived EMF of *T. congolense* IL3000, and the transgenic EMF successfully underwent metacyclogenesis *in vitro*. The newly constructed expression vector pSAK was designed for integration into the α - β tubulin locus, which is tandemly arranged in the *T. congolense* genome. The expression cassette of pSAK/enhanced green fluorescent protein (EGFP) was transfected into the EMF by electroporation. An EMF expressing EGFP was successfully generated and differentiated into an MCF that constitutively expressed EGFP. The *in vitro* metacyclogenesis system in combination with the transgenic EMF technique will be important tools to investigate the molecular mechanisms of metacyclogenesis.

Oral presentation II (Day 1: 11:00-11:50 on September 13)

O-4

Molecular biological tools for the immunization against *Theileria parva*

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East Coast Fever (ECF) is a devastating tick-borne disease in African cattle caused by the parasite *Theileria parva*. Due to its unusual biology, it has been extremely difficult to control *T. parva*. The most optimal solution would be a recombinant vaccine but the immune responses against this parasite are quite complex. Although it is known that *T. parva* specific CD8⁺ cytotoxic T lymphocyte (CTL) responses are fully protective, the discovery of the responsible antigens remains a challenging task. Generating a significant protective CTL response hasn't been possible using DNA-based, live vector or subunit vaccines.

We have constructed and tested several different vaccination protocols based on the polymorphic immunodominant molecule (PIM), an antigen of the parasite and on the Bm86, a concealed antigen of the vector. The work on the last protein is still in progress though we have finished already three experiments with PIM. The first two consisted of several sequential DNA immunizations with PIM whilst in the third a heterologous prime boost using DNA followed by a hepatitis B core-PIM fusion protein boost was applied containing parts of the latter protein. In the two first experiments, significant responses were detected in the 30% of the animals. In the third experiment, antibody responses were detected, but nevertheless, animals showed no CTL or CD4⁺ T cell proliferation and all animals died after challenge with no difference between controls and immunized animals. As a result, we have started to analyse the possible reasons of lack of immunologic responses in the vaccinated cattle. Preliminary experiments show that DNA vaccination seems to be more challenging than when compared to several other animals. For this reason we have also constructed a vaccine using *Listeria monocytogenes* as a carrier for our proteins. Hopefully it will help to solve the problems concerning DNA vaccination in cattle. The significance of these findings for the development of vaccines against *T. parva* will be discussed during the oral presentation.

Oral presentation II (Day 1: 11:00-11:50 on September 13)

O-5

Gene silencing in *Echinococcus multilocularis* protoscoleces using RNA interference

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In this study, we investigated the potential of gene silencing in *Echinococcus multilocularis* protoscoleces using RNA interference (RNAi). As the method for introduction of siRNA, firstly, soaking and electroporation were examined their effects on the viability of the protoscoleces and their efficacy on siRNA introduction using fluorescent labeled negative control siRNA. Among the several conditions of those two methods, electroporation with 100 V-800 μ F condition showed the best results, revealing > 80% viability of treated protoscoleces and effective introduction of fluorescent labeled siRNA. This designed electroporation was then evaluated its ability to induce RNAi on the level of mRNA and protein expression and the effect on the viability of protoscoleces using siRNAs targeting 14-3-3 and elp. Consequently, the two target mRNAs showed reduction to $21.8 \pm 2.5\%$ and $35.5 \pm 0.4\%$ in the 14-3-3- and elp-siRNA-treated protoscoleces respectively, compared to untreated controls on day 3. Moreover, siRNA-treated protoscoleces showed significant reduction on target proteins at day 15, 14-3-3 protein was reduced to $22.1 \pm 7.1\%$, while two proteins of elp were reduced to $68.7 \pm 10.4\%$ and $26.2 \pm 1.8\%$ compared to untreated controls. Reductions of viability to $58.0 \pm 23.0\%$ and $55.1 \pm 14.6\%$ were observed in 14-3-3- and elp-siRNA-treated protoscoleces respectively on day 15, while untreated controls showed $98.4 \pm 1.4\%$ and electroporation controls showed $83.0 \pm 2.5\%$ viability; although ANOVA analysis showed no significant differences. In conclusion, we successfully demonstrated RNAi mediated knock-down of target gene expression in *E. multilocularis* protoscoleces on both transcriptional and translational level. The method will be a powerful tool for investigating gene function and identifying essential gene products of the parasite.

Oral presentation II (Day 1: 11:00-11:50 on September 13)

O-6

Comparison of vaccine efficacy of tetraspanin3 (TSP3) protein by subcutaneous and intranasal immunization

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Echinococcus multilocularis causes an important zoonotic cestode disease, which progresses as a tumor-like proliferation in the liver of intermediate hosts-human and rodents. Tetraspanin (TSP) is a transmembrane protein abundant in the tegument or body wall of *Echinococcus* and has been proved to be a vaccine candidate against echinococcosis when subcutaneously administrated. Mucosal immunization induces both local and systematic immune response in many cases. In this study, TSP1 and TSP3 were evaluated for the vaccine efficacy against echinococcosis by intranasal administration. The result showed TSP3 has a 62.1% liver cyst reduction which is greatly higher than that for TSP1 (37.1%). Subsequently, TSP3 was used to comprise the immune response and vaccine efficacy between subcutaneous and intranasal immunization. The results showed that the cyst reduction of TSP3 vaccinated subcutaneously and intranasally vis-à-vis non-vaccinated mice were 81.9% and 61.3% respectively. All the results above suggested that although TSP3 has different protective effect against primary alveolar echinococcosis between two different administration, this study should be a opening of the study of tetraspains' applications in different infective stages of *Echinococcus* by different administration.

Oral presentation III (Day 1: 14:45-16:10 on September 13)

O-7

The ambivalent role of type-I-interferon in rabies virus immunosubversion and pathogenesis

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Rabies virus (RABV) - a strictly neuronotropic Rhabdovirus transmitted by the bite of an infected animal- is responsible for over 55,000 human death per year. RABV has developed an immunosubversive strategy funded on the destruction of migratory CD8⁺T cells through the up-regulation of neural B7-H1, an interferon (IFN) dependent gene. Thus, the moderate inflammation and IFN response in the nervous system (NS) that characterized RABV infection might be important for RABV immunosubversive strategy. The innate immune sensor RIG-I is the sensor of RABV infection. We found that overexpression of LGP2, an inhibitor of RIG-I signalling, blocks RABV-mediated IFN response *in vitro*. To impair innate immune response and elucidate whether and how the IFN response contributes to RABV immunosubversion, we compared RABV pathogenesis and immune parameters triggered in the infected NS of WT and a new transgenic mice model overexpressing LGP2 (hLGP2 mice). After an intramuscular RABV injection, the IFN response was not detected in hLGP2 mice NS, confirming the appropriateness of the model. The hLGP2 mice exhibited maintenance of CD8⁺T cells in their brain that correlates with their ability to clear RABV off their brain and their reduced morbidity compare to WT. CD8⁺T cells protection in hLGP2 NS was associated with the absence of neural B7-H1 induction indicating that impairment of IFN response is sufficient to weaken RABV immunosubversive. These results demonstrated that IFN response plays a key role in RABV pathogenesis. To further study the role of type-I-IFN in RABV pathogenesis, RABV neuroinvasiveness was compared in WT and type-I-IFN-receptor deficient (IFNR^{-/-}) mice. Two days post-infection (pi), higher RABV transcription level was detected in IFNR^{-/-} than in WT spinal cord whereas 4 and 7 days pi RABV infection was similar in the two types of mice, suggesting that type-I-IFN slows down RABV accessibility to the NS but does not control further RABV neuroinvasiveness. Despite similar amounts of RABV transcripts in their brain, IFNR^{-/-} mice died earlier than WT mice, indicating that the type-I-IFN response may be important to prevent neuronal dysfunction. From this work we conclude that the IFN response during RABV infection is, in a certain extent, essential to set up an efficient immunosubversive strategy and also to prevent neuronal disorders.

Oral presentation III (Day 1: 14:45-16:10 on September 13)

O-8

Rabies Control Activities in Central Province, Sri Lanka

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In Sri Lanka, Rabies deaths have decreased dramatically and are now in the stage of being eliminated at the District level. In order to achieve the rabies-free environment in the region, more effective rabies control programs and research works should be implemented continuously.

Hokkaido University has been involved in the rabies control projects in Central Province, Sri Lanka since 2005 under the 21st Century COE program on Zoonosis Control and the Global COE program on "Establishment of International Collaboration Centers for Zoonosis Control". With close communication with our counterparts in the University of Peradeniya, the only higher institution nationwide providing education for veterinary surgeons, we are currently working on laboratory and epidemiological research and relevant training toward the elimination of rabies in Central Province as well as in the country.

Laboratory work has been undertaken since the establishment of "Rabies Control Unit" in the University of Peradeniya in September 2005. Funded by the 21st Century COE program, the new laboratory has significantly contributed to decentralizing the capabilities of diagnosing suspected rabies animals in the nation and to offer training opportunities for young veterinary surgeons to improve their laboratory techniques as well as management skills of animal rabies control.

In parallel, several field epidemiological studies have been conducted in collaboration with universities, local and federal health government, and community representatives. The studies included two surveys in urban and rural areas to assess the level of knowledge, attitude, and practice among general population and health-seeking behaviors of animal bite victims. A dog ecological survey was also conducted to trace canine behaviors within or around communities. In order to show to what extent the Information and Education Campaign (IEC) materials are cost-effective for prevention, awareness leaflets were introduced to pet and non-pet owners and subsequent preventive educational opportunities for pupils were integrated into their school curricula. These comprehensive studies compiled the range of knowledge for building future rabies control strategies as well as enhanced the effectiveness of overall rabies control programs toward eliminating rabies in the District and Province level.

Oral presentation III (Day 1: 14:45-16:10 on September 13)

O-9

Alterations in membrane architecture in Flavivirus-infected cells

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Flaviviruses are a group of positive-sense RNA viruses that include major human pathogens such as yellow fever virus (YFV), West Nile virus (WNV), and dengue virus (DENV). Like other RNA viruses that infect mammalian, insect and plant cells, flaviviruses induce dramatic changes in the membrane architecture of the infected cell. These membrane alterations aid the viruses in replication and assembly of progeny virions. Furthermore, flavivirus particles are enveloped, which means that they contain a lipid bilayer as an essential part of their virion structure. DENV infects about 50 million humans per year. Mosquitoes transmit the virus and viral replication within the arthropod vector is required for transmission to a new host. Infection of both human and mosquito cells with DENV results in enhanced synthesis of new lipid derived structures and extensive membrane rearrangements. Recently, the three-dimensional organization of DENV-induced membranes has also been imaged using electron tomography. Despite considerable knowledge of the virus life cycle remarkably little is known about the interactions between virus and host that involve viral and cellular membranes. It is also unknown whether the human host and mosquito vector have similar requirements for membrane lipid composition and architecture to ensure optimal viral replication and transmission.

We are pursuing a systematic analysis of the precise role of lipids in DENV infection. We observe a clear difference between the overall lipid compositions of DENV-infected human cells compared to uninfected cells. These observations are also consistent in mosquito cells suggesting a conservation of membrane and lipid requirements between the disease vector and the host. Efforts are currently being focused on identifying lipid-mediators and signaling events important for a productive DENV infection. These studies are also being extended to analyze the lipid environment of DENV-infected *Aedes aegyptii* mosquitoes. Preliminary data from these approaches will be discussed with a focus on host-pathogen interactions. Through these studies we hope to systematically identify the lipid and protein mediators of signaling events that drive intracellular ultra structural alterations upon DENV infection. The knowledge of lipid metabolism and structures induced following cellular perturbations such as virus infections will be important for a greater understanding of the influence of the environment on biological membranes. Most importantly, these studies will facilitate comparison of both human and mosquito lipid and protein environments for their response to virus infection. Primarily, it will identify the differential requirements of DENV as it shuttles between the invertebrate and vertebrate environments during its replication cycle.

Oral presentation III (Day 1: 14:45-16:10 on September 13)

O-10

Chondroitin sulfate-E enhances Japanese encephalitis virus infection in neuronal cells

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Proteoglycans are major components of the cell surface and the extracellular matrix, which are composed of glycosaminoglycans (GAGs) and core proteins. GAGs are long unbranched polysaccharides consisting of a repeating disaccharide unit, each of which can be sulfated in variable position and quantities, and have diverse functions in the body. Several studies have suggested the relationship between GAGs and viral infection, and have found that soluble GAGs inhibit certain viral infection. In this study, we found soluble CS-E (sCS-E) enhanced JEV infectivity in a neuronal cell line (neuro2A). Although sCS-E inhibited the binding of JEV to cell surface, it eventually resulted in enhancement of viral infection through interruption of JEV-induced interferon (IFN) synthesis. In neuro2A, shRNA knockdown of N-acetylgalactosamine 4-sulfate 6-O-sulfotransferase, which synthesizes CS-E from CS-A by catalyzing transfer of sulfate, resulted in the reduction of the susceptibility to JEV. In 17-day-old rats (JEV resistant), intracerebral administration of CS-E with JEV led to significantly increased viral load as compared with mock-treatment. These results imply that brain CS-E may be a host factor involved in the susceptibility of neurons to JEV.

Abstract

Oral presentation III (Day 1: 14:45-16:10 on September 13)

O-11

Bovine and Murine Macrophage degradation of scrapie and BSE PrP^{Sc}

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[Backgrounds]

Transmissible spongiform encephalopathies (TSEs), also known as prion diseases, are progressive neurodegenerative disorders that typically exhibit long incubation periods prior to the onset of clinical symptoms. TSEs include scrapie in sheep and goat, bovine spongiform encephalopathy (BSE) in cattle, chronic wasting disease (CWD) in deer and elk, and Creutzfeldt-Jakob disease (CJD) in humans. The causative agents of TSEs are thought to be the disease specific abnormal prion protein (PrP^{Sc}) that is formed from the host-encoded cellular prion protein (PrP^C). The recent appearance of variant CJD (vCJD) has raised public health concerns that BSE may be transmissible to humans across the species barrier via dietary exposure of BSE-contaminated foods

One prominent feature of TSEs is the absence of a host immune response. However, the exposure of TSE agents does not always give rise to disease symptoms or the accumulation of PrP^{Sc} in the host. Actually, a large number of cattle have been fed PrP^{Sc}-contaminated food, the vast majority has not been diagnosed with BSE. The reasons underlying why the cattle fed the same food did not develop TSE remain unclear. There might be numerous defense and clearance mechanisms that inhibit TSE agents in the host. Following oral exposure, TSE agents firstly accumulate within the gut-associated lymphoid tissue (GALT). Macrophages within the gut tissue may play an important role as an initial host responder against PrP^{Sc}. However, the precise roles of macrophages in the elimination of PrP^{Sc} have not been thoroughly investigated. In this study, we focused on the macrophages that play a role in the prevention of exogenous agents in the early infectious stages.

[Results]

PrP^{Sc} within mouse scrapie- and BSE- affected brain homogenates were degraded sequentially in the bovine and murine macrophages when investigated by immunoblotting and confocal microscopy. To determine the infectious activity of PrP^{Sc} in macrophages, macrophage lysates were intraperitoneally inoculated into mice. The mice with degraded brain homogenates in macrophage demonstrated prolonged incubation times. PrP^{Sc} degradation was also significantly influenced in the presence of lysosomal and proteasomal inhibitors in macrophages. PrP^{Sc} in macrophages was colocalized with lysosomal markers and ubiquitin as observed by confocal microscopy. These findings indicated that PrP^{Sc} was generally degraded by both the lysosomal and proteasomal pathways in macrophages.

[Conclusion]

We provide direct evidence that macrophages are able to generate progressive and efficient degradation of PrP^{Sc} in TSE-affected brain homogenates. This findings suggested that animals possess biological protective systems against PrP^{Sc}. PrP^{Sc} may be degraded through lysosomal and proteasomal pathways in macrophages, although the exact molecular mechanism underlying these pathways remain unsolved. Further studies are required to determine how PrP^{Sc} is degraded during the cellular trafficking and re-cycling systems in host cells.

Oral presentation Day 2: September 14

Oral presentation IV (Day 2: 9:15-10:25 on September 14)

O-12

Cell entry of Lassa virus

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The arenavirus Lassa virus (LASV) is endemic to West Africa and infects several hundred thousand individuals each year with thousands of deaths. There is currently no vaccine available and therapeutic intervention is limited resulting in a mortality of 15-30% among hospitalized Lassa fever patients, making LASV arguably one of the most neglected tropical pathogens. Virus cell entry is the first step of every viral infection and represents a promising target for therapeutic intervention that allows blocking the pathogen before it can take control over the host cell for replication. Upon attachment to its cellular receptor, LASV enters the cell by an unusual route that is independent of clathrin- and caveolin and delivers the virus to acidified endosomes bypassing classical routes of incoming vesicular trafficking. Our present project aims at the characterization of the cellular mechanisms of LASV entry and the identification of cellular factors involved. We uncovered that LASV entry into prototypic human cells involves microtubular transport to late endosomes and depends on sorting in the host cells multivesicular body (MVB)/late endosome involving the endosomal sorting complex required for transport (ESCRT). Productive infection of LASV depended in particular on the lipid phospholipid lysobisphosphatidic acid (LBPA) and the ESCRT proteins Tsg101 and Vps4, which are normally involved in sorting of membrane receptors into intraluminal vesicles of the MVB. The ESCRT-associated protein Alix was identified as a negative regulator of LASV entry. In sum, our results indicate that, upon endocytosis, LASV passes through the endosomal sorting pathway of the host cell at the level of the MVB/late endosome. Efficient virus infection depends on sorting of the virus-receptor complex into intraluminal vesicles by the ESCRT, suggesting that LASV uses a pathway normally involved in receptor degradation.

Oral presentation IV (Day 2: 9:15-10:25 on September 14)

O-13

Hantaviruses and Arenaviruses coexistence in different geographic regions of Argentina

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New World hantaviruses and arenaviruses are associated with American rodents of the subfamily Sigmodontinae; some of them are etiologic agents of hantavirus pulmonary syndrome (HPS) and hemorrhagic fever, respectively. In Argentina, the hantavirus and arenavirus infections are characterized by a wide geographic distribution as well as a high genetic diversity. In northwestern provinces, Orán, Bermejo, and Laguna Negra (LN) hantaviruses are etiologic agents of HPS; recently, Latino (LAT) arenavirus was isolated, for the first time in Argentina, from *C. callosus*, the same rodent reservoir for LN hantavirus. In the central region, Lechiguana, Maciel, and Pergamino hantaviruses co-circulate with Junín (etiologic agent of Argentine Hemorrhagic Fever), Oliveros, and LCM arenaviruses. Here we report the first evidence of natural co-infection of *C. callosus* rodents with LN hantavirus and LAT arenavirus, in northwestern Argentina. Serum samples from *C. callosus* were screened for hantavirus and arenavirus infection by detection of IgG antibodies by ELISA. Lung tissue samples from hantavirus positive rodents were examined by RT-PCR for arenavirus and hantavirus; PCR products were sequenced. From a total of 223 serum samples from *C. callosus* examined by ELISA, 39 were positive: 23 for arenavirus, 14 for hantavirus, while two were positive for both antigens. LN hantavirus sequences were obtained from: 16 *C. callosus*; LAT arenavirus sequences were obtained from one *C. callosus*; finally, both LN hantavirus and LAT arenavirus sequences were obtained from 3 *C. callosus*. Both LN hantavirus and LAT arenavirus infections are maintained in *C. callosus* rodent host population in northwestern Argentina. While some individuals are infected with a single virus, LN or LAT-like, others are co-infected with both of them. Recombination among arenaviruses from different lineages and genetic reassortment among hantaviruses may occur in nature. The potential of these viruses to exchange genomic material provide a basis for the emergence of new viruses.

Oral presentation IV (Day 2: 9:15-10:25 on September 14)

O-14

Hantavirus nucleocapsid protein promotes cis-Golgi targeting of glycoprotein Gc

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Hantaviruses belonging to the family *Bunyaviridae* are important rodent-borne zoonotic pathogens causing hemorrhagic fever with renal syndrome and hantavirus pulmonary syndrome. The genome of Hantavirus is composed of three segmented negative-strand RNA encoding nucleocapsid (N) protein, glycoproteins Gn and Gc, and RNA-dependent RNA polymerase. The virion is thought to be formed in the Golgi complex, but the process leading to the particle formation remains unclear. In this study, to understand the transport mechanism of viral proteins, the intracellular localizations of the Gn and Gc proteins were investigated. In the Hantaan virus (HTNV)-infected Vero E6 cells, the Gn and Gc proteins were localized to cis-Golgi. When the Gn and Gc proteins of HTNV were expressed by plasmid vector, the Gn protein was localized to cis-Golgi, whereas the Gc protein tended to disperse into cytoplasm, although a part of Gc protein was localized to cis-Golgi. However, the extent of the cis-Golgi targeting of Gc protein was significantly increased by co-expression of the Gn, Gc and N proteins. N proteins of other hantaviruses, such as Seoul virus, Puumala virus and Sin nombre virus, also supported the cis-Golgi targeting of HTNV Gc protein heterologously. The analysis of the truncated HTNV N proteins showed that amino acid position 1-30 and 116-155 in N protein contributed to the function. These results suggest that Hantavirus N protein is important for the promotion of the cis-Golgi targeting of Gc protein.

Oral presentation IV (Day 2: 9:15-10:25 on September 14)

O-15

Persistent infection of Puumala virus in Syrian hamsters (*Mesocricetus auratus*) resembling hantavirus infection in natural hosts

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[Introduction]

Hantaviruses cause severe human illnesses, such as hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS). In contrast to human infection, hantaviruses do not cause any symptoms in host rodents and persist for a long period. However, the mechanisms mediating persistent infection in natural hosts remain unknown because of the lack of animal model mimicking hantavirus infection in the natural host. In this study, we report that Syrian hamsters have a high susceptibility to infection of Puumala virus (PUUV), a causative agent of HFRS, and the infected hamsters maintain PUUV for a long period.

[Materials and methods]

Subadult (4 weeks old) and adult (8 weeks old) hamsters were inoculated subcutaneously with 3,300ffu of PUUV Sotkamo strain. Blood and tissue samples are collected from 3 to 70 days post infection (dpi). Serum samples were used for the measurement of antibody responses, and tissue samples were used for the TaqMan quantitative RT-PCR analysis and pathological analysis.

[Results]

Although both of IgM and IgG antibodies to PUUV were produced in subadult and adult hamsters, IgM responses in subadults were stronger and longer than that in adults. Neutralizing antibody responses in both age groups were almost equivalent. Viral RNA load in lungs peaked on 14 days in subadult hamsters, and the viral RNA persisted for 70 days. The viral RNA was also detected in kidney, spleen, liver, heart, and brain. In adults, viral RNA loads in various organs were much lower than those of subadults. In subadult hamster, the viral antigen was detected in lung, kidney, cerebellum and adrenal gland. Slight inflammatory reactions were observed in lung, cerebellum and adrenal gland. On the other hand, no antigen and any inflammatory reaction were observed in adult hamsters.

[Discussion]

Viral RNA persistence in the existence of high level and prolonged antibodies is the characteristic feature of PUUV infection in Syrian hamsters, which is quite similar to the hantavirus infection in the specific rodent host. Therefore, PUUV infected hamsters, especially subadults, could be useful model to clarify what factors contribute to hantavirus persistence in rodents. In addition, hamsters showed high susceptibility to PUUV infection. Therefore, this model can be used to evaluate the efficacy of vaccines and antiviral drugs.

Oral presentation V (Day 2: 13:30-14:55 on September 14)

O-16

Insights into the 3D structure and assembly of influenza virus by cryo-electron microscopy and hybrid methods: New views of an old virus

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Influenza virus remains a global health threat, with millions of infections annually and the impending threat that a strain of non-human influenza may develop into a human pandemic. This has been recently exemplified by the current H1N1 pandemic virus of swine origin. Despite its importance as a pathogen, little is known about the virus structure, in part because of its intrinsic structural variability (pleiomorphy). 3D structures of both symmetrical and non-symmetrical viruses alone and in complex with neutralizing antibodies or Fabs can be obtained by electron microscopy. Thus, electron microscopy is uniquely positioned to provide structural information on the structure and antigenic potential of viral antigens. In this talk I will discuss the use of various 3D reconstruction techniques in electron microscopy coupled with molecular modeling to gain insights into the 3D structure and assembly of influenza virus. Notably, obtained 3D structures of influenza virions distinguish two kinds of glycoprotein spikes [hemagglutinin (HA) and neuraminidase (NA)] in the viral envelope, resolve the matrix protein layer lining the envelope, and depict internal configurations of ribonucleoprotein (RNP) complexes. Future prospects of using electron microscopy to aid vaccine development will be discussed as will the use of like methods to study other viruses such as HIV.

Oral presentation V
(Day 2: 13:30-14:55 on September 14)

O-17

Recognition of the influenza virus hemagglutinin by neutralizing antibodies

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Current influenza vaccines provide protection only against viral isolates similar to the vaccine strain, and will likely prove ineffective against an emerging pandemic virus, such as the swine-origin H1N1 viruses that arose in early 2009. In contrast, antibodies recognizing conserved epitopes in the major surface antigen, hemagglutinin, neutralize across multiple virus subtypes. Here we present structural and mechanistic data highlighting the interaction of several monoclonal antibodies with the hemagglutinin. Such antibodies have immediate clinical applications and should guide the design of improved vaccines that elicit similar, broadly neutralizing antibodies.

Oral presentation V
(Day 2: 13:30-14:55 on September 14)

O-18

Potency of the A/2009 (H1N1) pandemic influenza vaccine prepared from an isolate of swine origin, A/swine/Hokkaido/2/1981

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The recent A/2009 (H1N1) pandemic influenza has spread over the world. As the preparedness for pandemic influenza, we have been conducting global surveillance of animal influenza, since influenza viruses of natural hosts, feral water birds, domestic poultry, and pigs have been involved in the emergence of pandemic influenza viruses. In the present study, an influenza virus strain, A/swine/Hokkaido/2/1981 (H1N1) (Sw/Hok/81) was selected as the A/2009 (H1N1) pandemic influenza vaccine strain from the library of H1N1 virus isolates from humans, pigs, and water birds. Sixteen H1N1 virus strains in our virus library (<http://virusdb.czc.hokudai.ac.jp/>) were used in the present study. These viruses were propagated in the allantoic cavity of 10-day-old chicken eggs at 35 °C for 48 hours. In antigenic characterization by hemagglutination-inhibition (HI) test, isolates from pigs including Sw/Hok/81 were closely related to the H1N1 pandemic influenza virus strain, A/Narita/1/2009 (H1N1) (Narita/2009). Sw/Hok/81 grew significantly more efficiently in embryonated chicken eggs compared to Narita/2009 and other isolates from pigs. Sw/Hok/81 was selected for the test vaccine strain on the basis of its antigenicity and its growth capacity in chicken embryos. For the vaccine preparation, the virus was purified from allantoic fluids by differential centrifugation and sedimentation through a 20-50% sucrose gradient and inactivated with 0.1% formalin. Female, 4-week-old, BALB/c mice were vaccinated twice by subcutaneous injection with 20µg, 4µg, and 0.8µg of the inactivated whole particle virus in 100µl of PBS at 2-week intervals. Two weeks after the second vaccination, these mice were challenged intranasally with 10^{5.7} PFU/ml of a pandemic strain, Narita/2009. The mice were monitored daily for their body weight for 14 days. Three days after the challenge, four mice were sacrificed to determine the virus titers in their lungs. The body weight losses of vaccinated mice were suppressed in a dose-dependent manner compared to mice inoculated with PBS instead of the vaccine and the test vaccine provided significant reduction in virus replication in the lungs. In the present study, inactivated whole virus particle vaccine for the A/2009 (H1N1) pandemic influenza virus was prepared from virus isolate from pig in Hokkaido, Japan. The present results suggest that influenza virus isolate from pig was ideal vaccine strain to the A/2009 (H1N1) pandemic influenza. Influenza virus isolates stocked in the library could be provided for vaccine strain since their pathogenicity, antigenicity, genetic information and yield in chicken embryos have already been characterized.

Oral presentation V
(Day 2: 13:30-14:55 on September 14)

O-19

Protective Effect of HLA-A*2402 restricted CTL-inducing peptides against Influenza A virus infection

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[Introduction] The current influenza virus vaccines induce antibodies that protect against antigenically related viral strains. They do not, however, protect against antibody-escape variants of seasonal influenza A viruses or new pandemic influenza A viruses emerging from non-human reservoirs. To develop a broadly protective influenza vaccine, we focused on cytotoxic T lymphocyte (CTL) because CTL is able to recognize conserved internal viral peptides presented by the class I MHC of infected cells as epitopes. However the HLA-restricted CTL immune system targets different epitopes in different individuals. To overcome HLA dependence, we focused on HLA-A*2402 because the gene frequency of HLA-A *2402 is relatively high in Asian populations, especially in the Japanese (approximately 60%). In this study, we designed HLA-A*2402 restricted CTL epitopes derived from H5N1 Influenza A virus internal proteins and evaluated their protective effect against Influenza A virus infection using HLA-A*2402 transgenic mice (A24Tg).

[Methods] HLA-A*2402 restricted CTL epitope peptides were predicted by CTL epitope peptide prediction programs from avian influenza virus strain A/Hong Kong/483/97(H5N1) internal proteins, and 48 high-scored peptides were selected and synthesized. To verify immunogenicity of the peptides, *in vivo* cytotoxicity assay was used. To evaluate protective effect of CTL-peptide vaccine in a human CTL immune system, A24Tg mice, in which a human CTL immune system have been reconstituted, were immunized intranasally with the selected peptides, and challenged with several different influenza A virus strains. After virus infection, the survival rate and the changes in body weight were daily monitored and lung virus titers at day 5 after virus infection were measured. In addition, to examine T cell induction effect of immunodominant peptides in lung, we performed an immunohistochemical analysis of lungs using anti-mCD3 (T cell marker) or anti-mCD8 (CTL marker) antibodies.

[Results and Conclusions] CTL-inducing peptide vaccine inoculated A24Tg mice survived after influenza A virus infection regardless of virus subtypes. Furthermore, body weight loss of immunized mice was not observed and lung virus titers at infection day 5 in the immunized groups were significantly lower than those of unimmunized groups. As a consequence of immunohistochemical analysis of the lungs, accumulation of mCD3 or mCD8-positive cells was only observed in intranasally immunized mice. This result suggested that direct induction of epitope specific CTL in lungs was required for complete protection. We have demonstrated that the Influenza A virus specific CTL without neutralizing antibody induction have protective effect against Influenza A virus infection regardless of virus subtypes. In this study, we established peptide-based CTL-inducing vaccine system with several computed epitope prediction programs. These results provide the basis of CTL-inducing Influenza vaccine development for human use.

Oral presentation V
(Day 2: 13:30-14:55 on September 14)

O-20

Control mechanism for the infiltration of inflammatory cells after influenza A virus infection

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After influenza A virus infection, a variety of inflammatory cells are recruited into the virus-infected sites as a host defense response, and an inflammatory response takes place. In particular, inflammatory cell infiltration is a critical step for this response, and this process is tightly coordinated by the interaction of cells with their surrounding extracellular matrix (ECM) proteins. In addition, matrix metalloproteinases (MMPs) and their inhibitors play an important regulatory role in the inflammatory response, and are also involved in a number of pathological processes, such as fibrosis, chronic inflammation, and tissue destruction. Thus alteration of the expression levels of these proteins may affect pathological condition after influenza A virus infection. However, the role of these molecules (ECMs, cell adhesion molecules, MMPs and MMP inhibitors) after the viral infection is still unclear. In this study, we found that the alteration of gene expression of several MMPs and ECMs in the lung of the mice infected with influenza A virus strains, PR/8 (A/Puerto Rico/8/34 (H1N1)). In particular, the expression of tissue inhibitor of metalloproteinase-1 (TIMP-1), which is a specific inhibitor of MMPs, was very strongly induced in the lung after PR/8 infection. It has been reported that an imbalance of MMP/TIMP expression ratios was implicated in pathological disorder such as pulmonary fibrosis. Therefore, it is suggested that the increased expression of TIMP-1 is correlated to the pathological condition after influenza A virus infection.

Oral presentation VI (Day 2: 15:15-16:05 on September 14)

O-21

Disinfectant-resistant mycobacteria

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Non-tuberculous *Mycobacterium* spp. are ubiquitous in the environment and cause infections and pseudo-infections in health care settings throughout the world. Among these, the rapidly-growing *Mycobacterium* (RGM) species, *M. abscessus* (subsp. *abscessus* and subsp. *massiliense*), *M. chelonae*, and *M. fortuitum* are particularly problematic due to their ubiquitous presence in hospitals' water sources and the difficulty of treating the infections they cause. There is increasing awareness of *M. abscessus* subsp. *abscessus* and *massiliense* in particular as emerging pathogens. Of particular concern is the increasing frequency with which glutaraldehyde-resistant RGM are being associated with nosocomial outbreaks, sometime reaching epidemic proportions as recently documented in Brazil. Glutaraldehyde (GTA) is the most widely used chemical disinfectant for medical devices in hospitals worldwide.

What is known of the mode of action of this disinfectant in other bacteria suggests that changes in the surface-exposed composition of the cell envelope resulting in decreased binding and/or penetration of GTA may be one of the mechanisms through which RGM develop high levels of resistance. Because of the important role played by the mycobacterial outer membrane in drug susceptibility and host-pathogen interactions, there is thus some concern that the widespread use of GTA and other related aldehyde disinfectants in clinical settings is impacting on the selection of resistant populations of RGM with possible consequences on cross-resistance to drugs and pathogenicity. In support of this assumption, we recently demonstrated that reduced porin expression was one of the mechanisms through which *M. smegmatis* and *M. chelonae* develop high levels of resistance to GTA and a further aldehyde disinfectant, ortho-phthalaldehyde (OPA). In part due to defects in porin expression, the GTA/OPA-resistant *M. chelonae* isolate under study also displayed unusually high levels of resistance to a number of drugs including rifampicin, ciprofloxacin, clarithromycin, erythromycin, vancomycin, tetracycline and linezolid.

We have assembled a collection of GTA-resistant isolates of *M. chelonae* and *M. abscessus* subsp. *massiliense* responsible for nosocomial infections and pseudo-infections in different regions of the world. Biochemical and genetic studies are in progress to investigate their molecular mechanisms of resistance to aldehyde-based disinfectants and determine the potential impact the phenotypic changes undergone by these strains might have had on their susceptibility to drugs and pathogenicity. The results of this study could lead to the implementation of optimized strategies for the disinfection of medical devices and suggest more efficient treatments to cure those nosocomial infections caused by disinfectant-resistant isolates.

Oral presentation VI (Day 2: 15:15-16:05 on September 14)

O-22

Epidemiological Study of Tuberculosis in Lechwe (*Kobus lechwe*) in Zambia and Anthroozoonosis between Human and Chimpanzee in Tanzania

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An African antelope, Lechwe (*Kobus lechwe*) inhabits Lochinvar National Park, Zambia, close to the capital city of Lusaka. Lochinvar NP is divided into two areas, on a fully protected area, and the other a game reserve (GR) for game hunting area. The game reserve is also used as pasturage by the indigenous people, so lechwe and livestock share the habitat. The game reserve has been known as a bovine tuberculosis (bTB) contaminated area since surveillance of tuberculosis in Lechwe for about forty years ago. A recent report revealed that 5.2% of the livestock in this area were bTB positive by the comparative intradermal tuberculin test. The agent of bTB, *Mycobacterium bovis*, is being maintained between lechwe and livestock in Lochinvar. Bovine tuberculosis infects not only bovidae, but also humans by way of contaminated meat or dairy products. Therefore, controlling bTB is very important for both livestock and human health. Previous reports mainly targeted the abundant species, lechwe, but little known about bTB infection in other wild bovidae animals. However, the other bovidae animals in Lochinvar, wilde beast, zebra, water buffalo, kudu etc., are also likely to be reservoirs. In this study, we survey the bTB in bovidae wild animals in Lochinvar NP by *Mycobacterium* isolation and DNA typing of the *Mycobacteria*. By comparing these results with the animals ecology, we predict the bTB mode of migration into Lochinvar NP.

The Chimpanzee is genetically and evolutionarily the closest species of primate to humans, and therefore, some immunological features similar to humans. Therefore, their susceptibility to human pathogens may also be high. Human disease transmission is a great concern for wild chimpanzee conservation. Recently, human Metapneumovirus and RS virus originating from humans were detected from sick chimpanzees, and these agents are possibly transmitted by tourists. Human borne diseases in wild chimpanzees are not well studied. Therefore, our study objective is to detect human borne diseases from habituated wild chimpanzees. Our study site, Mahale Mountains National Park, is located in the west end of Tanzania, where one group of chimpanzees is habituated to humans and are often watched by tourists. Three epidemic outbreaks have been recorded in Mahale, but the cause of infection was not determined. Conducting timely sampling in the wild is difficult because no one can predict the outbreak. Therefore, we will collect present data of chimpanzee diseases while we are waiting for pathogenic disease outbreaks. We will also conduct serological test of human pathogens on domestic chimpanzees for estimation of the species to which chimpanzees are highly susceptible. Results in both the wild and captive chimpanzees will be compared in the future.

O-23

Whole-genome analysis of an attenuated *Ehrlichia ruminantium* vaccine strain using next-generation sequencer

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A rickettsial bacterium *Ehrlichia ruminantium* is the causative agent of heartwater of ruminants throughout sub-Saharan Africa and the Caribbean. The disease causes substantial livestock losses in endemic areas and is recently recognized as an emerging zoonosis. *E. ruminantium* Gardel strain, which was originally isolated as a highly virulent strain in Guadeloupe (French West Indies), was previously attenuated by serial passages in mammalian cells for the use as a live attenuated vaccine. Here, we determined the genome sequences of virulent and attenuated Gardel strains for genome-wide comparison using massively parallel sequencing technology. Comparative genome analysis revealed that there are only 16 genomic changes observed between virulent and attenuated strains, including 1 large deletion, 5 SNPs and 10 1-bp deletions/insertions (indels). The attenuated strain had an 896-bp deletion in the region crossing *map1-2* and *map1-3* genes, encoding an outer membrane protein MAP1 (Major Antigenic Protein 1), which resulted in merger into a single gene. Three out of five SNPs were nonsynonymous and created amino acid substitutions in three different genes such as *mutS*, *hupB*, and *pyrH* genes. In addition, 10 indels, mainly located in homopolymeric tracts of As or Ts, affected 5 different genes including *proP* and 4 other genes encoding hypothetical proteins with unknown functions. These findings might provide novel insights into genetic basis of *E. ruminantium* virulence and will thus contribute to a rational design of future vaccines.

Program Poster Session

- P-1 Comparison of the pathogenesis of Seoul virus infection in experimentally infected laboratory rats and naturally infected wild rats**
Shumpei P. Yasuda (Dept. of Microbiology, Graduate School of Medicine, Hokkaido University)
- P-2 A new model of Hantavirus infection in SCID mice**
Takaaki Koma (Dept. of Virology, Graduate School of Medicine, Hokkaido University)
- P-3 Efficient Isolation Method for Puumala Hantavirus by Using Syrian Hamsters**
Takahiro Seto (Lab. of Public Health, Dept. of Environmental Veterinary Sciences, Graduate School of Veterinary Medicine, Hokkaido University)
- P-4 Establishment of enzyme-linked immunosorbent assay for epidemiological studies of hantavirus infection in Mexico**
Ngonda Saasa (Lab. of Public Health, Dept. of Environmental Veterinary Sciences, Graduate School of Veterinary Medicine, Hokkaido University)
- P-5 Biological significance of cleavage of Filovirus glycoprotein (GP) by furin**
Masahiro Kajihara (Dept. of Global Epidemiology, Research Center for Zoonosis Control, Hokkaido University)
- P-6 Enzyme-linked immunosorbent assay for the detection of filovirus species-specific antibodies**
Eri Nakayama (Dept. of Global Epidemiology, Research Center for Zoonosis Control, Hokkaido University)
- P-7 Different potential of C-type lectin-mediated entry between Marburg virus strains**
Keita Matsuno (Dept. of Global Epidemiology, Research Center for Zoonosis Control, Hokkaido University)
- P-8 Molecular epidemiology of rabies virus in Zambia**
Walter Muleya (Dept. of Molecular Pathobiology, Research Center for Zoonosis Control, Hokkaido University)
- P-9 Detection of polyomavirus in wild rodents**
Yasuko Orba (Dept. of Molecular Pathobiology, Research Center for Zoonosis Control, Hokkaido University)
- P-10 Characterization of Japanese encephalitis virus infection in CSM14.1 cells**
Megumi Okumura (Dept. of Molecular Pathobiology, Research Center for Zoonosis Control, Hokkaido University)
- P-11 Functional analysis of murine flavivirus resistance gene *Oas1b***
Kanako Moritoh (Lab. of Laboratory Animal Science and Medicine, Dept. of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University)
- P-12 Molecular characterization of expressed sequence tags for *Dermanyssus gallinae* for the development of a novel control method of the mite**
Masayoshi Isezaki (Lab. of Infectious Diseases, Dept. of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University)
- P-13 Isolation and characterization of serine proteases of tsetse and stable flies, and their roles in the differentiation of bloodstream forms of *Trypanosoma brucei* into procyclic forms**
Satoshi Miyazaki (Dept. of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University)
- P-14 Comparative analysis of pathogenicity of the Philippines isolates of *Trypanosoma evansi***
Hirohisa Mekata (Lab. of Infectious Diseases, Dept. of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University)
- P-15 Molecular detection and characterisation of tick-borne protozoan parasites in indigenous zebu cattle and wild bovids along wildlife-livestock corridors in Kenya**
Naftaly Githaka (Dept. of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University)
- P-16 A schizont-derived protein, TpSCOP, is involved in the activation of NF- κ B in *Theileria parva*-infected lymphocytes**
Kyoko Hayashida (Dept. of Education and Collaboration, Research Center for Zoonosis Control, Hokkaido University)
- P-17 Characterization of *Theileria orientalis putative hemolysins***
Joseph Kamau (Dept. of Education and Collaboration, Research Center for Zoonosis Control, Hokkaido University)
- P-18 Experimental visceral leishmaniasis in alymphoplasia (*aly/aly*) mice**
Saruda Tiwananthagorn (Dept. of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University)
- P-19 Identification and epidemiological study of intestinal *Tritrichomonas foetus* infection in cats in Japan**
Junko Doi (Dept. of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University)
- P-20 Simultaneous detection of canine taeniids by Reverse line blotting**
Maria Teresa Armua-Fernandez (Dept. of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University)

- P-21 Characterization of the interaction of influenza virus NS1 with Akt.**
Mami Matsuda (Division of Cancer Biology, Institute for Genetic Medicine, Hokkaido University)
- P-22 The role of cyclooxygenase-2 (COX-2) in suckling mice infected with H3N2 subtype influenza virus**
Yuji Sunden (Lab. of Comparative Pathology, Dept. of Veterinary Clinical Sciences, Graduate School of Veterinary Medicine, Hokkaido University)
- P-23 Amino acid change Glu to Lys at 627 of the PB2 was responsible for the activation of viral transcription**
Naoki Yamamoto (Lab. of Microbiology, Dept. of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University)
- P-24 H9N2 avian influenza virus acquires high pathogenicity by the introduction of a pair of di-basic amino acid residues at the hemagglutinin cleavage site and consecutive passages in chickens** Kosuke Soda (Lab. of Microbiology, Dept. of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University)
- P-25 Characterization of influenza viruses isolated from domestic and feral birds in Vietnam**
Naoki Nomura (Lab. of Microbiology, Dept. of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University)
- P-26 Studies on the pathogenicity of influenza virus in chicken**
Saya Kuribayashi (Lab. of Microbiology, Dept. of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University)
- P-27 Antigenic analysis of current H5N1 highly pathogenic avian influenza viruses and efficacy of an inactivated vaccine prepared from non-pathogenic virus isolates** Shintaro Shichinohe (Lab. of Microbiology, Dept. of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University)
- P-28 Genetic Characterization of Influenza A Viruses Isolated from Wild Waterfowl in Zambia**
Edgar Simulundu (Dept. of Global Epidemiology, Research Center for Zoonosis Control, Hokkaido University)
- P-29 Predicting of the Antigenic Change of the Pandemic (H1N1) 2009 Influenza Virus Hemagglutinin**
Manabu Igarashi (Dept. of Global Epidemiology, Research Center for Zoonosis Control, Hokkaido University)
- P-30 Effect of IFN-alpha/beta signal on immune response against influenza virus infection**
Osamu Noyori (Dept. of Global Epidemiology, Research Center for Zoonosis Control, Hokkaido University)
- P-31 The role of antibody in heterosubtypic protective immunity against influenza virus infection (plan)**
Mieko Muramatsu (Dept. of Global Epidemiology, Research Center for Zoonosis Control, Hokkaido University)
- P-32 Experimental study on antibody-mediated heterosubtypic immunity against influenza virus infection**
Daisuke Tomabechi (Dept. of Global Epidemiology, Research Center for Zoonosis Control, Hokkaido University)
- P-33 Molecular characterization of immunoinhibitory factors PD-1/PD-L1 in bovine leukemia virus-infected cattle**
Ryoyo Ikebuchi (Dept. of Disease Control, Graduate School of veterinary Medicine, Hokkaido University)
- P-34 Amino acid substitutions or insertion in the Meq proteins could affect their transactivation and transformation abilities**
Shiro Murata (Dept. of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University)
- P-35 Prevalence and Carrier Status of Leptospirosis in Smallholder Dairy Cattle and Peridomestic Rodents in Kandy, Sri Lanka**
Chandika D. Gamage (Dept. of Global Health and Epidemiology, Graduate School of Medicine, Hokkaido University)
- P-36 Behavioural pattern of rural residents of Kandy, Sri Lanka in relation to leptospirosis: implications for intervention**
Chinyere Nwafor-Okoli (Dept. of Global Health and Epidemiology, Graduate School of Medicine, Hokkaido University)
- P-37 Functional analysis of an α -helical antimicrobial peptide derived from a novel mouse defensin-like gene**
Akira Kawaguchi (Dept. of Molecular Pathobiology, Research Center for Zoonosis Control, Hokkaido University)

Abstract Poster Presentation

P-1 Comparison of the pathogenesis of Seoul virus infection in experimentally infected laboratory rats and naturally infected wild rats

Shumpei P. Yasuda, Rika Endo, Kenta Shimizu, Takaaki Koma, Erdenesaikhan Tegshduuren, Vu Dinh Luan, Kumiko Yoshimatsu, Vu Thi Que Huong and Jiro Arikawa

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The natural reservoir of Seoul virus (SEOV) is the Norway rat (*Rattus norvegicus*), which is established as a laboratory rat. To study the ecology of SEOV, we examined antibody titers, amounts of virus genome copies and mitogenic responses of splenocytes in infected laboratory rats and wild rats.

Male and female laboratory rats (WKAH, 6 weeks old) were experimentally infected with SEOV strain SR-11 (6.0×10^4 ffu/rat, intra-peritoneal route). Lungs, sera and spleens were collected at periodic intervals. Fifty-four *R. norvegicus* rats were captured in Hai Phong Port and Saigon Harbor in Vietnam, where SEOV was endemic. IgM and IgG antibody titers to SEOV were measured using the ELISA method. The amounts of virus genome copies in lungs were examined using a real-time PCR method. The mitogenic responses of splenocytes were evaluated using concanavalin A.

In laboratory rats, IgM antibody to SEOV appeared 6 days post inoculation (dpi) and IgG antibody appeared 9 dpi. The IgM antibody titers began to decrease 13 dpi, whereas the IgG antibody titer increased until 16 dpi. The amount of virus genome peaked at 6 dpi and then decreased to a low level. Maximum amount of virus genome was about 700,000 copies/mg in lungs. In wild and laboratory rats, the amount of virus genome in males were larger than those in females. In wild rats, 8 (80%) of 10 IgM- and IgG-positive rats, 8 (57%) of 14 IgG-positive rats and 2 (6.7%) of 30 antibody-negative rats were positive for the viral genome in lungs. No virus genomes were found in two females. One of them was IgG-positive and the other was IgM- and IgG-positive. The amounts of virus genome were about 1,000~10,000,000 copies/mg in lungs. Geometric means of copy numbers were about 510,000 for IgM- and IgG-positive rats and 53,000 for IgG-positive rats. In laboratory rats, mitogenic response of the splenocytes to concanavalin A were detected. No differences of mitogenic responses were detected between non-infected and infected (16 dpi and 27 dpi) rats.

Genetic, serologic and immunological characterization confirmed persistent infection of SEOV in wild rats and transient infection in laboratory rats. The transient increase of virus genome in laboratory rats indicated a different pathogenesis from that in naturally infected rats. Further immunological studies are necessary to compare the pathogenesis in natural infection and experimental infected rats.

P-2 A new model of Hantavirus infection in SCID mice

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[Introduction] Hantavirus causes two important rodent borne viral zoonoses, hemorrhagic fever with renal syndrome (HFRS) in Eurasia and hantavirus pulmonary syndrome (HPS) in North and South Americas. Both hantavirus infections are commonly characterized by thrombocytopenia, renal failure especially in HFRS and pulmonary edema especially in HPS. Because hantaviruses does not affect the cytopathic *in vitro*, it has been hypothesized that the pathogenesis of hantavirus infection would be the immunopathological mechanisms. However, because there is no practical disease model to study either HPS or HFRS, its detailed mechanisms remain largely unknown. In the present study, we tried to establish a new hantavirus disease model in SCID mice.

[Materials and Methods] Six to seven weeks-old C.B-17 SCID mice and BALB/c mice were inoculated with Hantaan virus cl-1 (HTNV). Spleen cells from HTNV-infected BALB/c mice (5×10^7 cells/mice) on 12 days post inoculation (dpi) or B-cells depleted fraction of the splenic cells (3.5×10^7 cells/mice) were transferred into HTNV-infected SCID mice on various dpi. Body weight, mortality, specific antibody, renal function, platelet count and histopathology of recipient mice were monitored. Two kinds of control experiments were carried out. One is HTNV-infected SCID mice without transfer and the other is non-infected SCID mice that received the spleen cells from non-immunized BALB/c mice.

[Results and Discussion] Rapid weight loss was observed around 5-day after transfer in SCID mice that received spleen cells from HTNV-infected BALB/c mice. However, almost all SCID mice were finally recovered. The SCID mice that received cells on 28 dpi finally died or were moribund, but HTNV-infected non-transferred SCID mice also died around 42 dpi without rapid weight loss. Hantavirus-specific antibody were detected from 2-day after transfer in SCID mice that received immune splenocytes. Neutralizing antibodies were also detected from 5-day after transfer in SCID mice that received immune splenocytes. Blood urea nitrogen (BUN) level for assessment of renal function were sequentially elevated from 8-day after transfer in SCID mice that received immune splenocytes. Elevation of BUN was not observed in infected SCID mice without cell-transfer and non-infected SCID mice that received the spleen cells from non-infected BALB/c mice. Therefore, these results suggest that renal dysfunction in this study occurred by immune splenocytes especially T or B cell. Decrease of platelet count was seen in both infected SCID mice with transfer and infected SCID mice without transfer. Therefore, immune splenocytes were not contribute to platelet count. Lung edema was histologically observed in SCID mice that received cells, but it was also observed in HTNV-infected without cell transfer. These results suggested that immune splenocytes does not largely contribute to lung edema.

To clarify the contribution of specific antibodies for recovery, B cell-depleted spleen cells were used. Basically similar results were obtained from this trial except following several points. In spite of the rapid weight loss around 6 days after cell-transfer, death after transfer were not observed. Hantavirus-specific antibody were not detected until 6-day after transfer and IFA titer were obviously lower than when transferred whole splenocytes. Because neutralizing antibodies were not detected, specific antibodies did not contribute to recover at least in this time. As higher elevation of BUN level was observed without transfer of B cells, which indicates that T cell associate with renal dysfunction.

In this model, we observed some clinical features common to HFRS and HPS. However, platelet count and lung edema were observed even in HTNV-infected SCID mice without transfer of cells. C.B-17 SCID mice have no functional T and B cells, but they alternatively possess higher NK activity and innate immunity. Therefore, mice without T, B and NK cells should be used as recipient mice in future.

P-3 Efficient Isolation Method for Puumala Hantavirus by Using Syrian Hamsters

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Introduction: Puumala hantavirus (PUUV) belongs to the genus Hantavirus within the family Bunyviridae and is the causative agent of hemorrhagic fever with renal syndrome (HFRS) in Europe and European Russia. This virus is difficult to isolate in cell culture. Therefore, biological properties of PUUVs different geographical origins have not been fully characterized. In this study, we developed the efficient system to isolate PUUV by the combination of enzyme-linked immunosorbent assay (ELISA) for hantavirus nucleocapsid protein (NP) and injection to Syrian hamsters. By using this system, we successfully isolated two PUUV isolates and analyzed genetic and antigenic properties of the isolates.

Material and Methods: Sixty-eight of *Myodes glareolus* were captured in Samara Oblast, Russia in 2005. PUUV NP was detected from lung homogenates of these rodents by NP-ELISA. Anti-PUUV antibody was detected from sera of these rodents by using indirect immunofluorescent antibody assay (IFA). Lung homogenates of NP-positive and seronegative *M. glareolus* were inoculated to 4-week male Syrian hamsters subcutaneously. Twelve days post inoculation (d.p.i.), all hamsters were sacrificed and lungs, kidneys, spleen, and serum samples were collected from these hamsters. Lung homogenates of NP-positive and seronegative hamsters were used for virus isolation in Vero E6 cells. Genetic and antigenic characterization of newly isolated PUUV was performed by comparison with other PUUVs. The ultrastructure of Vero E6 cells infected with new isolates was observed by transmission electron microscopy.

Results: Three out of 68 *M. glareolus* were NP-positive and seronegative. Lung homogenates of these 3 rodents were inoculated to Syrian hamsters. Virus isolation in cultured cells was attempted by inoculation of lung homogenates of NP-positive hamsters to Vero E6 cell monolayers. As a result, two strains of PUUV (strain samara49/CG/2005 and samara94/CG/2005) from *M. glareolus* were isolated in Vero E6 cells. Nucleotide and amino acid sequences of these isolates were quite similar to a PUUV sequence from HFRS patient in Samara (F-s808) with 96.7-99.3 % and 99.3-100.0 %, respectively. Antigenic properties of these isolates were similar to other Russian and Finnish PUUVs. Morphologic features of strain Samara49/CG/2005-infected Vero E6 cells were quite similar to Hantaan hantavirus.

Conclusion: Selection of samples from NP-positive and seronegative rodents and inoculation of the samples into Syrian hamsters are an efficient strategy for isolation of PUUV. The strategy may be also applied to other hantavirus for virus recovery and isolation.

P-4 Establishment of enzyme-linked immunosorbent assay for epidemiological studies of hantavirus infection in Mexico

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Introduction

Hantaviruses in the Americas are important causative agents of hantavirus pulmonary syndrome (HPS) in humans with about 40% fatality. A previous survey indicated that several apparently distinct hantaviruses are circulating in Mexican wild rodents. Therefore, this study was carried out to establish more efficient diagnostic methods in order to obtain more epidemiological information on hantavirus infections in Mexico.

Materials and Methods

Hantavirus (Montano virus; MTNV) S gene was amplified by reverse transcriptase polymerase chain reaction (RT-PCR) from a lung tissue of Aztec mouse, *Peromyscus aztecus* captured in Guerrero State, Mexico in 2006 and cloned into bacterial expression vector pET43.1b(+). The recombinant hantavirus nucleocapsid protein (rNP) was expressed from the transformed *E. coli* and purified with Nickel-chelating resin method. Similarly, rNP of Sin Nombre virus (SNV), which causes HPS in United States of America, was also prepared. Two hundred rodent sera obtained from Guerrero State, Mexico in May 2007 were analyzed by enzyme-linked immunosorbent assay (ELISA) and western blotting (WB) using the two rNPs to detect hantavirus antibodies. RT-PCR was carried out on S RNA gene extracted from respective rodent lung tissues followed by direct sequencing.

Results

Fourteen out of 200 (7%) rodent sera analyzed by ELISA were strongly positive for hantavirus antibodies with more than 2.0 optical densities. All ELISA positive sera were confirmed by WB analysis. RT-PCR detected hantavirus S RNA segment gene in lung tissues of 13 of the 14 sero-positive rodents. Sequencing of the open reading frame (ORF) showed that the positive rodents were infected by MTN virus. All rodents positive for hantavirus were of genus *Peromyscus*.

Discussion

This Mexican hantavirus rNP could detect hantavirus antibodies in all the sera detected with SNV rNP antigen. The rNP of Mexican MTNV could therefore be useful and reliable in diagnostic and epidemiological investigations of hantavirus infections in Mexico. The results also show that infections are common in genus *Peromyscus* rodents. Detection of viral RNA in spite of the existence of high level of antibodies in 13 out of 14 sero-positive rodents indicates that most of the sero-positive *Peromyscus* mice were persistently infected with MTN virus.

Since MTNV is a genetically distinct hantavirus, the antigenicity still needs further characterization using monoclonal antibodies. In addition, isolation of the Mexican virus would further facilitate antigenic characterization by neutralization tests. Until now, the importance of human hantavirus infections in Mexico still remains to be investigated.

Abstract Poster Presentation

P-5 Biological significance of cleavage of Filovirus glycoprotein (GP) by furin

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Ebolavirus (EBOV) and *Marburgvirus* (MARV) are filamentous, enveloped, and nonsegmented negative-strand RNA viruses in the family *Filoviridae*. They cause lethal hemorrhagic fever in human and nonhuman primates. Filoviruses possess a single surface glycoprotein (GP) which mediates viral entry. Hence, it is thought that GP is associated with viral growth, host specificity, tissue tropism, and pathogenicity. Proteolytic cleavage of surface proteins is a prerequisite for many viruses to infect host cells. Although it is known that filovirus GPs are cleaved by an ubiquitous subtilisin-like endoprotease, furin into two subunits, GP1 and GP2, previous studies showed that the GP cleavage was not essential for viral infectivity in cultured cells and pathogenicity in rhesus macaques. However, since a furin recognition motif (Arg-X-Arg/Lys-Arg) is highly conserved among GPs of all known filoviruses, it has been suggested that the GP cleavage has some biological significance in the filovirus life cycle.

We produced vesicular stomatitis viruses (VSVs) pseudotyped with wild-type (WT) or uncleaved (Cl(-)) GP of five species of EBOV and two strains of MARV, which have amino acid substitution in a furin recognition motif. Then, we tried to verify some hypotheses for biological significance of the GP cleavage. First one was that the GP cleavage is required to infect some particular animals including natural reservoir hosts. To examine this hypothesis, infectivities of the pseudotyped VSVs were compared in various cultured cells derived from bats, primates, rodents, canine, and swine, all of which are known to be susceptible to filoviruses. In this experiment, no significant difference in infectivities was observed between WT and Cl(-) GP-pseudotyped VSVs, suggesting that the GP cleavage is not essential for filoviruses to infect these cells. Second hypothesis was that the GP cleavage might be important for some particular entry pathway such as C-type lectin-mediated entry. To examine this hypothesis, infectivities of pseudotyped VSVs with WT or Cl(-) GP were compared in K562 cells expressing C-type lectins, such as DC-SIGN and hMGL. However, pseudotyped VSVs with Cl(-) GP showed enhanced infectivity as well as those with WT GP, indicating that the GP cleavage is not necessary for C-type lectin-mediated entry. Thirdly, there was a possibility that structural change as a result of the GP cleavage influences efficiency of GP processing by cathepsin, a host protease in endosome, followed by membrane fusion. To test this hypothesis, infectivities of the viruses were analyzed in the presence of cathepsin inhibitors. This analysis showed no difference in infectivities among the viruses, suggesting that WT and Cl(-) GPs had a similar susceptibility to the processing by cathepsin.

In conclusion, the GP cleavage was not essential for filoviruses to infect any cultured cells tested in this study and to use C-type lectins for their entry and had no influence on susceptibility of GP to cathepsin. To reveal the biological significance of GP cleavage, analyses of infectivity in Vero E6 cells modified with various chemicals, for example, which inhibit endocytosis and intracellular transport, are in progress. Furthermore, we will focus on not only viral entry step but also other GP functions, such as its involvement in virus budding and immune evasion.

P-6 Enzyme-linked immunosorbent assay for the detection of filovirus species-specific antibodies

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Several enzyme-linked immunosorbent assays (ELISA) for the detection of filovirus-specific antibodies have been developed. However, diagnostic methods to distinguish antibodies specific to the respective species of filoviruses, which provide the basis for serological classification, are not readily available. We established an ELISA using His-tagged secreted forms of the transmembrane glycoproteins (GPs) of five different Ebola viruses (EBOV) species and one Marburg virus (MARV) strain as antigens for the detection of filovirus species-specific antibodies. The GP-based ELISA was evaluated by testing antisera collected from mice immunized with virus-like particles, as well as humans and nonhuman primates infected with EBOV or MARV. In our ELISA, little cross-reactivity of IgG antibodies was observed in most of the mouse antisera. Although sera and plasma from some patients and monkeys showed notable cross-reactivity with the GPs from multiple filovirus species, the highest reactions of IgG were uniformly detected against the GP antigen homologous to the virus species that infected individuals. We further confirmed that MARV-specific IgM antibodies were specifically detected in specimens collected from patients during the acute phase of infection. These results demonstrate the usefulness of our ELISA for diagnostics as well as ecological and serosurvey studies.

P-7 Different potential of C-type lectin-mediated entry between Marburg virus strains

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The surface glycoprotein (GP) of filoviruses is responsible for virus entry into cells. It has been shown that GP interacts with cellular C-type lectins for virus attachment to cells. Since primary target cells of filoviruses such as macrophages, dendritic cells, and hepatocytes express C-type lectins, C-type lectin-mediated entry is thought to be a possible determinant of virus tropism and pathogenesis. We compared the efficiency of C-type lectin-mediated entry between two Marburg virus strains, Angola and Musoke, by using a vesicular stomatitis virus (VSV) pseudotype system. VSV pseudotyped with Angola GP (VSV-Angola) infected K562 cells expressing the C-type lectins, hMGL or DC-SIGN more efficiently, than VSV pseudotyped with Musoke GP (VSV-Musoke). Unexpectedly, there was no strong evidence to indicate that the binding affinity of the C-type lectins to GPs correlated with the different efficiency of C-type lectin-mediated entry. Accordingly, the mucin-like region, which contains a large number of carbohydrate chains and principally interacts with the C-type lectins, was not essential for the difference. Site-directed mutagenesis identified the amino acid at position 547, which converted the efficiency of C-type lectin-mediated entry. A three-dimensional model of Angola GP revealed that this amino acid was in close proximity to the putative site of cysteine protease cathepsin processing. Interestingly, inhibitors of cathepsins B and L reduced the infectivity of VSV-Angola less efficiently than that of VSV-Musoke in the C-type lectin-expressing K562 cells, whereas only a limited difference was found in control K562 cells. The amino acid at position 547 was critical for the different effects of the inhibitors on the virus infectivities. These results suggest that the efficiency of C-type lectin-mediated entry of filoviruses is controlled not only by binding affinity between C-type lectins and the carbohydrates on GP but also by some mechanisms underlying endosomal entry such as proteolytic processing by the cathepsins.

P-8 Molecular epidemiology of rabies virus in Zambia

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Based on the report by the national livestock epidemiology and information center (NALEICS) in Zambia, over 2100 and 4800 cases of dog and human rabies occurred from 2005-2009. In this study, the lineage of Zambian rabies virus strains was revealed by the use of phylogenetic analyses based on the nucleoprotein (NP) and glycoprotein (GP) gene.

A total of 150 IHC positive specimens stored at the university of Zambia, school of veterinary medicine from 1999-2009 were collected for this study. Of the 150 specimens, RNA was extracted from 87 specimens and out of which only 35 RNA samples were positive on RT-PCR for both the NP and GP genes. Phylogenetic analysis based on the NP gene showed two groups of strains of RABV circulating in Zambia. One group contained only strains from Zambia while the other included strains from Zambia, Tanzania and Mozambique, classified as Africa 1b. Phylogenetic analyses of the GP gene showed that the closest ancestor to the strains from Zambia was Africa 1a strains. The results indicated that the endemic strains in Zambia from 1999-2009 are classified into the Africa 1b lineage present in eastern and southern Africa.

Abstract Poster Presentation

P-9 Detection of polyomavirus in wild rodents

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Introduction

Members of the family *Polyomaviridae* are nonenveloped virus with a circular double-stranded DNA genome. At present, 5 human and 17 non-human polyomavirus species are known. Most mammalian polyomaviruses cause subclinical infections with life long persistence in their natural immunocompetent hosts. In general, it is believed that mammalian polyomaviruses have a narrow host range. However, there is a possibility that wild animals are a natural reservoir of several new polyomaviruses which have been recently discovered in humans. Most non-human polyomaviruses have been identified as contaminants in cell culture or laboratory animals. There are only a few reports about identification of polyomavirus in wild animals. It is necessary to investigate known or unknown polyomavirus infection in wild animals to discuss the possibility that polyomaviruses of animals can be transmitted to other species including humans and thereafter cause disease. In this study, we studied the presence of polyomavirus in wild rodents.

Methods

A total of 100 rodents were collected from Namwala, Lusaka and Mfuwe district in Zambia. DNA samples were extracted from spleen tissues of rodents. To detect polyomavirus genome, we performed nested broad-spectrum PCR using degenerated PCR primers for amplification of the regions encoding the viral capsid protein VP1 and VP3. The entire viral genome was obtained by inverse PCR, and subcloned into pCR4-TOPO for sequencing or pUC19 for amplification. Phylogenetic analysis was performed between the amino-acid sequences of viral proteins of polyomaviruses. To test whether the polyomavirus genome produce progeny viruses in culture cells, the linearized entire viral genome was transfected into various cell lines.

Results

We detected polyomavirus genome from a rat sample (1 of 100 rodents), and subsequently sequenced the entire viral genome of 4,899 base pairs. This viral genome appeared to have potential ORFs for the capsid proteins VP1, VP2 and VP3, and the early proteins small t antigen and large T antigen. Phylogenetic analysis revealed that it is novel member of *Polyomaviridae*. We detected transient expression of viral mRNA and proteins of VP1 and large T antigen in some cell lines transfected with the cloned entire viral genome, but we could not find productive infection with the novel polyomavirus in cell lines.

P-10 Characterization of Japanese encephalitis virus infection in CSM14.1 cells

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Newborn rats younger than 12 days old show 100% mortality by intracerebral inoculation of Japanese encephalitis virus (JEV), although no lethality is observed in rats older than 15 days of age. Previous studies have suggested the correlation of JEV-susceptibility with the level of neuronal differentiation and maturation. However, the detailed mechanism of the age-dependent resistance to JEV infection has remained unclear. Rat neuronal CSM14.1 cell line was established by immortalization of primary rat embryonic mesencephalic neural cells with a temperature-sensitive mutant of the simian virus 40 large T antigen. Several studies have suggested that CSM14.1 cells can be differentiated *in vitro* by elevating incubation temperature from 31-33°C to 37-39°C.

In this study, we compared the susceptibility of CSM14.1 cells cultured at 31°C (undifferentiated [undiff] cells) with that at 37°C (differentiated [diff] cells) to JEV and determined which steps of viral infection are responsible for the difference in susceptibility of CSM14.1 cells to JEV. Changing the culture temperature from 31 to 37°C induced rapid reduction within 3 days in the susceptibility of CSM14.1 cells to JEV infection and this was associated with increased expression of neuronal markers. In the infectious recovery assay that detects endocytosed viral particles, the number of internalized JEV within cells was higher in undiff cells than that in diff cells, although there was no significant difference in the amount of JEV bound to the cell surface between both cells. In innate immune reactions against JEV infection, the expression levels of mRNAs of IFN- α 1, IFN- β 1, and Oas1b in diff cells were higher than those in undiff cells at 48 hours post-infection.

These data suggest that JEV-susceptibility of CSM14.1 cells is rapidly decreased by neuronal differentiation and less susceptibility of diff cells is due in part to decrease in the degree of viral internalization. More efficient induction of IFN- α 1, IFN- β 1, and Oas1b genes in diff cells after JEV infection may play a role in viral clearance at a later step. CSM14.1 cells could be a convenient *in vitro* model system for investigating the interactions of JEV with neurons.

P-11 Functional analysis of murine flavivirus resistance gene *Oas1b*

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Type1 interferons (IFNs) (IFN α/β) are produced and secreted from virus-infected cells and render the surrounding cells to induce a number of cellular proteins, including the Oas (Oligoadenylate synthetase). In mice, the *Oas* locus locates on Chr 5 and is composed of the *Oas* gene cluster, consisting of *Oas1a-h*, *Oas2*, and *Oas3*. Among these genes, *Oas1b* has been identified as a flavivirus-resistant gene, of which protein product, Oas1b confers resistance to flaviviruses including West Nile virus (WNV) in mice. Most strains of laboratory mice such as C57BL/6J (B6), BALB/c, and DBA/2 lost the functional *Oas1b* gene during selective breeding. Therefore, laboratory mice are susceptible to flavivirus, whereas wild mouse-derived strains are known to be resistant to flavivirus due to the possession of the intact *Oas1b* gene.

In previous study, we generated *Oas*-congenic strain, in which the *Oas* locus of the MSM/Ms strain was introduced into the most widely used strain, C57BL/6J mice. *Oas*-congenic strain showed higher antiviral specificity than C57BL/6J parental strain possessing the same genetic background. However, *Oas*-congenic mice did not show resistance to influenza virus infection.

These data suggest that murine Oas1b proteins inhibit flavivirus multiplication specifically; however, the mechanism of it remains unknown. To explore the mechanism of Oas1b function, we established stably *Oas1b*-expressing cells, that the mouse *Oas1b* expression vector was introduced into BHK 21 cells. Then, we evaluated inhibitory activity of murine Oas1b to flavivirus replication using WNV replicon. The cells expressing Oas1b exhibited the reduction of WNV replicon replication. These data suggest that murine Oas1b affects flavivirus replication step inside the cell.

P-12 Molecular characterization of expressed sequence tags for *Dermanyssus gallinae* for the development of a novel control method of the mite

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The red mite, *Dermanyssus gallinae* is distributed worldwide, and an economically important parasitic pest of domestic chickens. The red mite could be responsible for the decrease in egg production, weight loss, and anemia, and the multiple infestation of red mite can cause the death of the host. In addition, red mite has been suggested as a potential vector of several pathogens. (e.g., *Salmonella*).

Chemical acaricide splaying is the current method generally employed for the control of the mite. Recently, the development of acaricide resistance in red mite suggests that alternative control methods are required. However, the genetic information of red mite is poorly known. Therefore, in this study, we performed a global analysis of gene expressions in red mite.

A plasmid cDNA library was constructed from the red mite collected from a poultry farm. A total of 432 expression sequence tags (ESTs) were sequenced from the plasmid cDNA library. When these sequences were compared to NCBI databases, 113 sequences were identified as the genes of known function.

Many of these clones have high-homology with the genes of *Ixodes scapularis*, known as a vector of the Lyme disease. In addition, one clone was found to be homologous to antioxidant, thioredoxin peroxidase which is suggested as a possible vaccine candidate for other ticks. Currently, functional analysis of these clones is in progress to identify new vaccine candidate and target molecule of acaricide. Moreover, the role of red mite as a vector is also analyzed for the transmission of various pathogens.

Abstract Poster Presentation

P-13 Isolation and characterization of serine proteases of tsetse and stable flies, and their roles in the differentiation of bloodstream forms of *Trypanosoma brucei* into procyclic forms

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Trypanosoma brucei, which causes a zoonotic African trypanosomiasis, is biologically transmitted by tsetse flies (*Glossina* spp.). When a tsetse fly sucks the mammalian blood containing the bloodstream forms (BSF), the parasites can differentiate into the insect forms (procyclic forms: PCF) in the tsetse midgut. Elucidation of mechanisms of PCF differentiation is hallmark for the noble strategies of control of trypanosomiasis. The treatment of BSF of *T. brucei* with a recombinant serine protease of *G. fuscipes* induced the PCF differentiation at 27°C in vitro (Abubaker et al., 2006). Therefore, serine protease(s) may play a role in triggering the PCF differentiation, but the molecular mechanism of the PCF differentiation remains unknown.

On the other hand, BSF of *T. brucei* does not differentiate into PCF in blood-sucking stable flies (*Stomoxys* spp.), although these flies can mechanically transmit some *Trypanosoma* species. The involvement of serine proteases of stable flies in the PCF differentiation has not been studied. Thus, the present study aims to isolation and characterization of serine proteases of tsetse and stable flies and examination of their roles in the PCF differentiation.

To date, 8 and 13 of partial sequences of serine proteases were identified from the cDNA of tsetse and stable flies, respectively. Some of them were highly similar with the *Gpl* sequence, suggested *Gpl* orthologs. Hereafter, isolations of the full-length transcripts of the *Gpl* orthologs by RACE-PCR, expressions of the genes using a bacterial expression system, and evaluations of the inducibilities of its recombinant proteins will be performed. In this seminar, based on results of these experiments, the roles of fly's serine proteases in PCF differentiation mechanism will be discussed.

P-14 Comparative analysis of pathogenicity of the Philippines isolates of *Trypanosoma evansi*

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Trypanosoma evansi, belonging to the subgenus *Trypanozoon*, often causes an severe wasting disease (Surra) of livestock and wild animals. This disease is endemic in Southeast Asia, Africa and South America, where thousands of animals die annually due to the disease. In Philippines, *T. evansi* is present in all 13 regions, and Surra is the second most important disease of livestock after fasciolosis. In recent years, highly virulent *T. evansi* has appeared and caused a severe disease with high mortality in livestock. However, little is known for the genetic factors responsible for the differences in virulence observed. Thus, to identify genetic factors related to the increase in the pathogenicity of highly virulent *T. evansi*, we determined whether difference in gene expressions are analyzed among the field isolates which show different pathogenicities from one another, isolated in the Luzon and Mindanao regions.

By the inoculation test using mice, the prepatent period and duration of survival varied significantly amongst isolates. To identify genes involved in the difference in virulence, subtraction cDNA library was constructed, and screened for cDNA expressed exclusively in the highly virulent or low virulent *T. evansi* in Philippines. The subtraction clones, gL2L3-01, which actively expressed in highly virulent isolates, and gL3L2-01, which actively expressed in low virulent isolates were found to encode proteins showing homology to the member of the trypanosome glycoprotein superfamily. Currently, these genes are insertionally over-expressed in a laboratory strain of *T. evansi*, and after that, we will study their virulence to animals to determine whether these gene products are determinants for the virulence or not.

P-15 Molecular detection and characterisation of tick-borne protozoan parasites in indigenous zebu cattle and wild bovids along wildlife-livestock corridors in Kenya

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Ticks and tick-borne diseases (TBDs) remain a huge obstacle to improved livestock production in Kenya, where millions of rural populations rely on small-scale mixed farming for their livelihoods. Encroachment of game parks and other wildlife habitats in the recent years have led to increased interaction between livestock and wildlife, possibly impacting on the transmission of TBDs. The piroplasms, comprising mainly the genera *Theileria* and *Babesia* cause some of the most debilitating TBDs, such as East coast fever and babesiosis, respectively. The distribution of these parasites closely follows that of their tick vectors, and hence the risk of infection with the corresponding TBDs. Many tick-borne parasites may infect multiple vector and mammalian host species, including wild animals, and consequently, mixed infections are not uncommon under field conditions. Currently, limited data is available on the contribution of wildlife to epidemiology of TBDs in Kenya.

In this study we determined infection rates of TBDs in indigenous cattle and wild bovids along livestock-wildlife corridors. The study also aims at characterizing parasite isolates identified from the mammalian hosts to establish genetic diversity at selected gene loci. Using semi-nested PCR assays with species-specific oligonucleotide primers for *Theileria parva*, we observed infection rates of 25-100 % in cattle, 50 % in Cape buffaloes, 26 % in Waterbucks and 100 % in Elands.

To determine the diversity of these parasites, we have selected two loci, the internal transcribed spacer (ITS) region of rRNA, and a segment of β -tubulin containing a highly variable intron. Preliminary ITS sequences of *T. parva* from cattle and buffalo isolates are largely similar, confirming previous studies. However, we found extensive sequence and size heterogeneity among *T. parva* isolates originating from cattle, buffaloes and elands at the intron located within the β -tubulin locus, perhaps indicating host adaptation for the parasite. Characterization of other parasites identified in this study is undergoing.

P-16 A schizont-derived protein, TpSCOP, is involved in the activation of NF- κ B in *Theileria parva*-infected lymphocytes.

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Theileria parva is an intracellular protozoan parasite that is transmitted by ticks and causes East Coast fever, a severe lymphoproliferative disorder in cattle in East and Central Africa. In the schizont stage, parasites reside in lymphocytes and induce uncontrolled proliferation of the host cell. In *Theileria*-transformed cells, the transcription factor NF- κ B is constitutively activated in a parasite-dependent manner, which is considered to be regulating host apoptotic pathway.

In the present study, TpSCOP was identified as an orthologous of the ToMRP, which is an erythrocyte Band3 binding protein expressed at the erythrocytic stage of a non-transforming *Theileria*, *T. orientalis*. TpSCOP was expressed at the surface membrane of the schizont stage of *T. parva*. In an attempt to identify the interacting molecule, TpSCOP was demonstrated to interact with F-actin. To investigate the potential role of TpSCOP in the cells, *TpSCOP* was introduced into mouse lymphocytes. In this cell line, the activation of NF- κ B and an increase in resistance to apoptotic signals were observed. The activation of MAPKs, including ERK and JNK, were also detected. Furthermore, the introduction of TpSCOP into *T. parva*-infected cells also enhanced the activation of NF- κ B.

This is the first report to demonstrate that parasite-derived molecule has the ability to activate the host NF- κ B pathway. Based on these results, TpSCOP likely plays a role in apoptosis inhibition during *Theileria* infection, possibly via changing the structure of host cell cytoskeleton.

Abstract Poster Presentation

P-17 Characterization of *Theileria orientalis* putative hemolysins

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Theileria are intracellular protozoan parasites of veterinary importance, and their impact on livestock production in many developing countries has a significant negative effect on the quality of human life. Host cell invasion is a crucial aspect of *Theileria* biology. To continue its life cycle, the schizont undergoes a differentiation and cellularization process to produce uninucleate merozoites, which are then liberated into the bloodstream where they invade erythrocytes. This process of invading more erythrocytes is repeated and in the process erythrocytes are lysed. It's not known which proteins are used to cause cell lyses leading to anaemia. To unravel this, we compared three genomes of *T. parva*, *T. annulata* and *T. orientalis*. Eight genes were predicted to encode polypeptides with hemolytic activity and designated as *Theileria* putative hemolysins (Tp-Hly). This is a class of predicted genes with potential as vaccine antigens for control of anemia due to *T. orientalis*. Out of the eight genes, three are soluble and rest transmembrane. We have successfully expressed two of the eight genes (Soluble and transmembrane). To confirm if the putative proteins have hemolytic activities, and or are involved in parasite-erythrocyte interactions, we have generated monoclonal antibodies for Tp-Hly colocalization by immunohistochemistry.

P-18 Experimental visceral leishmaniasis in alymphoplasia (*aly/aly*) mice

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Relapses to visceral leishmaniasis (VL) are frequent in immunocompromised humans and dogs in which *L. donovani* or *L. infantum* persists in spleen, bone marrow, as well as lymph nodes even after treatment. Experimental VL of mice with *L. donovani* results in the contrasting outcomes of infection in liver and spleen due to the organ-specific immunity. Liver is the site of acute resolving infection, whereas spleen becomes the site of parasite persistence. However, a role of lymph node in experimental VL has not been fully understood. We therefore employed alymphoplastic NF- κ B inducing kinase mutant (*aly/aly*) mice possessing lacking lymph nodes and Peyer's patches and structural abnormalities of spleen and thymus as a model in this study. Intravenous inoculation of *aly/aly* and *aly/+* (control) mice with 5×10^7 *L. donovani* promastigotes was conducted and parasite burdens, liver histology, cytokine/chemokine responses and number of FoxP3⁺ T cells of liver were analyzed. We demonstrated that the parasite burden was less in *aly/aly* mice in the early phase of infection (4-wk post-infection; WPI). However, the parasites remained in the liver of *aly/aly* mice at 12-WPI, whereas the most of parasites were removed in the *aly/+* mice. Impairment of granuloma formation and retention of infected cells in the liver were also demonstrated in *aly/aly* mice. Accordingly, higher parasite DNA was detected in the spleen, bone marrow, and peripheral blood of *aly/aly* mice at 12-WPI. Furthermore, RT-PCR/qPCR revealed the lower mRNA levels of cytokines/chemokines, including IP-10, MCP-1, IFN- γ , TNF- α , and iNOS in the liver of *aly/aly* mice, comparing with the control mice at 4-WPI. Interestingly, the mRNA level of FoxP3 in the liver of *aly/aly* mice was higher than those of *aly/+* over the course of infection. Only CD4⁺FoxP3⁺ T cells but not CD8⁺FoxP3⁺ T cells were determined in liver of both mice after *L. donovani* infection. In addition, concordance with the FoxP3 mRNA level, the numbers of CD4⁺FoxP3⁺ T cells in the liver of *aly/aly* mice were also higher than those of *aly/+* mice over the course of infection. In summary, defects of NF- κ B pathway and/or the lymph nodes may elucidate the noticeable paradoxical responses; including resistant in the early and susceptible in the late phase of *L. donovani* infection in *aly/aly* mice.

P-19 Identification and epidemiological study of intestinal *Tritrichomonas foetus* infection in cats in Japan

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Recently, it was discovered that *Tritrichomonas foetus* is a causative agent of intractable chronic large bowel diarrhea in cats. Many researchers had attributed that the disease to *Pentatrichomonas hominis*, a low pathogenic trichomonad capable of infecting the intestinal tracts of various mammalian hosts including cats. Feline trichomonosis are mainly caused by *T. foetus* and not *P. hominis* (Levy et al., 2003).

T. foetus (Riedmüller, 1928) is well known as a cattle pathogen in the genital organ and cause abortion, but the parasite is synonym of *Tritrichomonas suis* (Gruby, 1843) which parasitizes in the nasal cavity, stomach, cecum, colon and occasionally small intestine of pigs (Lun et al., 2005).

Feline *T. foetus* infection has been found in many countries and the prevalence was 14-31% in Europe and the U.S. However, no report was published from Japan.

In 2009, a case of feline trichomonosis was diagnosed at Hokkaido University animal hospital. The parasite was identified as *T. foetus* by nested PCR amplification of the ribosomal RNA gene with *T. foetus*-specific primers. In Japan, *P. hominis* is a common trichomonad in dogs. The number of anterior flagella is different in the precise observation, but general morphology of *T. foetus* is similar to *P. hominis* by ordinary microscopic examination. We isolated the parasite from the cat and confirmed the identification of the parasite by the precise observation and the DNA sequence.

Thus, We started an epidemiological survey of feline trichomonosis using cultivation and PCR methods.

One hundred forty-six feline fecal samples were submitted for this study. Thirty-eight samples were collected at the animal hospital of Hokkaido University. 58 samples were collected at a local animal hospital of Saitama prefecture. Forty-eight samples were collected at a local animal hospital of Hokkaido prefecture.

Twelve of the 146 samples (8.2%) were positive for *T. foetus*; three of the 97 samples (3.1%) were positive using culture procedure, five of the 59 fecal samples (8.4%) were positive using PCR procedure and seven of the 58 cultivated samples (12.1%) were positive using PCR methods.

Positive cats were between the ages of 1 month and 7 years. Three of 12 positive cats were reported to have colonic diarrhea. Six of positive cats were bred and six were mixed breed. Positive pure bred cats included American shorthair (3), Abyssinian (2) and Scottish Fold (1).

Our study indicates that feline *T. foetus* infection is spreaded in Japan, regardless bred and age. Thus, *T. foetus*-specific test (cultivation or PCR test) should be considered in cats with chronic diarrhea, those in which traditional diagnostic and treatments for other causes of feline diarrhea have failed to reach a clinical resolution.

P-20 Simultaneous detection of canine taeniids by Reverse line blotting

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Taeniid cestodes are distributed worldwide and responsible for human illness as well as condemnation of tons of carcasses and offal every year. Canids such as dogs, dingoes, foxes, etc. are the definitive hosts of important species of *Echinococcus* and *Taenia* meanwhile, livestock, rodents and humans serve as intermediate hosts. Unlike other parasitic diseases, taeniid infections cannot be discriminated by faecal egg detection methods; due to the similar morphological characteristics of the eggs among the species. Therefore, development of accurate diagnostic methods for epidemiological studies is essential. Reverse line blotting (RLB) assay is a hybridization method which can detect and differentiate several pathogens simultaneously on a single membrane. The aim of this study is to develop a RLB capable of detect and differentiate taeniid cestodes in canids. Firstly, we designed species-specific oligonucleotide probes (S-SONP) targeting NADH dehydrogenase subunit 1 (ND1) gene for differentiating 3 *Echinococcus* spp. (*E. granulosus* G1 genotype, *E. multilocularis* and *E. vogeli*) and 5 *Taenia* spp. (*T. crassiceps*, *T. hydatigena*, *T. multiceps*, *T. ovis* and *T. taeniaeformis*). Additionally; two common probes for *Echinococcus* genus and cestodes were designed. ND1 of the above mentioned parasites were PCR-amplified, blotted on a membrane and hybridized with the S-SONP. As a result, each S-SONP specifically bound with the corresponding taeniid ND1 gene. The experiment suggested that this RLB assay could be promising diagnostic method for epidemiological survey of taeniid infections in canids. Further studies using taeniid eggs isolated from dog faeces are conducted.

Abstract Poster Presentation

P-21 Characterization of the interaction of influenza virus NS1 with Akt.

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Avian influenza viruses belong to the genus influenza A virus of the family Orthomyxoviridae. The influenza virus consists of eight segmented minus stranded RNA that encode 11 known proteins. Among the 11 viral proteins, NS1 (non-structural protein 1, encoded on segment 8) has been implicated in the regulation of several important intra-cellular functions. In this report, we investigated the functional interaction of NS1 with serine threonine kinase Akt, a core intra-cellular survival regulator. In co-immunoprecipitation assays and GST pull-down assays, NS1 directly interacted with Akt. The interaction was mediated primarily through the Akt-PH (Pleckstrin Homology) domain and the RNA-binding domain of NS1. NS1 preferentially interacted with phosphorylated Akt, but not with non-phosphorylated Akt. Functionally, the NS1-Akt interaction enhanced Akt activity both in the intra-cellular context and in in vitro Akt kinase assays. Confocal microscopic analysis revealed that phosphorylated Akt interacted with NS1 during the interphase of the cell cycle predominantly within the nucleus. Finally, mass spectrometric analysis demonstrated the position at Thr215 of NS1 protein is primary phosphorylation target site through Akt activation. The results together supported the functional importance of influenza virus NS1 with Akt, a core intra-cellular survival regulator.

P-22 The role of cyclooxygenase-2 (COX-2) in suckling mice infected with H3N2 subtype influenza virus

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Influenza virus-associated encephalopathy (IAE) is rare and a fatal disease of human infants, and the medication of antipyretic (inhibitor of cyclooxygenase) has been suspected as a risk factor. Cyclooxygenase (COX) is an enzyme that catalyzes the production of prostanoids. COX-2 is one of the isoform of COX and inducible enzyme at inflammation sites. Previous report (Carey *et al.*, 2005) indicates that COX-2 expression is involved in the survive of influenza virus infected adult mouse. In this study, the role of COX-2 expression in influenza virus infection was pathologically investigated using COX-2 knockout suckling mice (COX-2^{-/-}).

Seven-days old suckling mice were inoculated H3N2 influenza virus (A/Aichi/2/68) through both nostrils. COX-2^{-/-} mice showed significantly lower mortality than wild type mice (COX-2^{+/+}). Histopathology of the lungs revealed diffuse and severe bronchiointerstitial pneumonia with COX-2-expressing macrophages in COX-2^{+/+} mice. Whereas in COX-2^{-/-} mice, the lung lesions were focal with infiltrations of neutrophils, macrophages and T-lymphocytes. Viral antigens were detected in the lungs of both mice by immunohistochemistry, and no significant difference in virus isolation titers. Blood plasma of COX-2^{-/-} mice contained significantly higher concentration of MCP-1, IL-12p70 and TNF-alpha than those of COX-2^{+/+} mice. Further, no brain lesion was observed in both mice.

The present results suggest that COX-2 expressing cells might lead the severe and diffuse tissue damage in the affected lungs in suckling mice. In human, hypercytokinemia of TNF-alpha and IL-6 has been postulated as the cause of IAE. Although some cytokines were upregulated in the blood plasma of COX-2^{-/-} mice, no brain histopathological change appeared in this study, suggests neither COX-2 deficiency nor following hypercytokinemia might be the primary cause of IAE in mouse model.

P-23 Amino acid change Glu to Lys at 627 of the PB2 was responsible for the activation of viral transcription

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Influenza viruses are enveloped and have segmented single-stranded RNA genome. The virus has eight negative-sense viral RNA (vRNA) segments which are packaged into viral ribonucleoprotein (vRNP) complexes. In the vRNPs, each vRNA segment is associated with the nucleoprotein (NP) and the RNA-dependent RNA polymerase. The viral RNA polymerase is a heterotrimeric complex of polymerase basic protein 1 (PB1), polymerase basic protein 2 (PB2) and polymerase acidic protein (PA). This complex is responsible for both transcription and replication of the vRNA genome. The polymerase complex binds to the host cell pre-mRNAs and snatches the 10-13 ribonucleotides containing Cap-structure by its endonuclease activity. These short capped RNA derived from host cell are then used as primers for the transcription of viral mRNAs. Each vRNA is synthesized through two steps. First, the polymerase complex synthesizes cRNA complementary to vRNA. Second, the polymerase complex synthesizes vRNA using cRNA as the template. However, the regulatory mechanisms of RNA synthesis are not well understood.

A/Hong Kong/483/1997 (H5N1) (HK/483) isolated from human causes lethal systemic infection in mice. In previous study, it was reported that the pathogenicity for mice decreased by substituting Lysine to Glutamic acid at position 627 of PB2. Additionally, it was shown that the substitution at position 627 of PB2 is involved in the regulation of viral growth *in vitro*. However, the details of molecular mechanisms were not clarified. Since the pathogenicity of influenza virus is dependent on the replication efficiency of the virus in the host, it is important to show the function of each of viral polymerase complex. The purpose of this study is to clarify the activation mechanism of viral transcription by the amino acid change at position 627 of HK/483 PB2. Now, it is analyzed whether the amino acid substitution of the PB2 influences on synthesizes of virus RNAs, vRNA, mRNA and cRNA.

P-24 H9N2 avian influenza virus acquires high pathogenicity by the introduction of a pair of di-basic amino acid residues at the hemagglutinin cleavage site and consecutive passages in chickens

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1. Background

Avian influenza by low pathogenic H9N2 viruses have occurred in poultry, resulting in serious economic losses in Asia and the Middle East. Its eradication is still difficult because of its low pathogenicity, frequently causing inapparent infection. It is important for the control of avian influenza to assess whether the H9N2 virus is capable of becoming pathogenic like H5 and H7 highly pathogenic avian influenza (HPAI) viruses. HPAI viruses possess HA with insertion of more than a pair of di-basic amino acid residues at the cleavage site, resulting in cleavage into HA1 and HA2 subunits by ubiquitous proteases such as furin and PC6. To investigate whether H9 virus can acquire high pathogenicity for chicken or not, a pair of di-basic amino acid residues was introduced into the cleavage site of the H9 and H5 HAs of non-pathogenic viruses. These mutant H9 and H5 viruses were then serially passaged in the air sacs of chick and their pathogenicity for chicken was assessed.

2. Materials and methods

We generated mutants of A/chicken/Yokohama/aq-55/2001 (Y55) (H9N2) (Y55) and A/duck/Hokkaido/Vac-1/2004 (Vac1) (H5N1) which have basic amino acid residues at their HA cleavage sites by site-directed-mutagenesis and reverse genetics. The amino acid sequences at the cleavage sites are as follows: Y55sub (H9N2) (PARKKR), Vac1sub (H5N1) (PRRKKR/G), and Vac1ins (H5N1) (PQRERRKKR/G). These mutant viruses were then consecutively passaged in the air sacs of three-day-old chicks. Passaged viruses were identified by their parental strains, mutation (substitution or insertion), and number of passages. For example, the designation Y55subP10 (H9N2) indicates that the amino acids at the HA cleavage site of the Y55 (H9N2) were substituted with basic amino acids as shown above, then passaged ten times in the air sac. Pathogenicity of the viruses was evaluated by inoculating into four-week-old chickens intravenously (1:10 diluted allantoic fluid, 200 μ l) or intranasally ($10^{6.5}$ EID₅₀/100 μ l).

3. Results

Over 75% of the chickens infected intravenously with Y55subP10 (H9N2) died, a rate of pathogenicity similar to that of HPAI viruses defined by OIE. On the other hand, all the chickens intranasally inoculated with Y55subP10 (H9N2) survived without showing any clinical symptoms, and the virus was detected in the restricted organs and tissues such as the respiratory tracts three days post infection. All chickens infected with Vac1subP3 (H5N1) and Vac1insP3 (H5N1) died by either inoculation procedures, and the viruses were recovered from systemic organs including the brain and the blood.

4. Conclusions

It was demonstrated that H9 influenza viruses have the potential to acquire intravenous pathogenicity for chicken although their morbidity via the natural route of infection is lower than that of H5 viruses. These results coincide with the fact that the HA subtypes of HPAI viruses are restricted to H5 and H7. Vac1subP3 (H5N1) and Vac1insP3 (H5N1), but not Y55subP10 (H9N2), were recovered from the brain and the blood after intranasal infection, indicating that hematogenous invasion of the virus to the brain is essential to exert their lethal pathogenicity for chicken. This notion is also supported by the result that Y55subP10 (H9N2) showed high pathogenicity for chicken only by intravenous inoculation, namely, direct injection to the blood vessels.

Abstract Poster Presentation

P-25 Characterization of influenza viruses isolated from domestic and feral birds in Vietnam

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[Introduction] Since late 2003, the highly pathogenic avian influenza virus of the H5N1 subtype has seriously affected poultry in Asia. Well over 500 million birds have died from infection or have been killed for control purpose. A highly pathogenic avian influenza virus is generated when a nonpathogenic virus brought in by migratory birds from nesting lakes in the north is transmitted to chickens via domestic ducks, geese, quails, turkeys, etc. Highly pathogenic avian influenza viruses have transmitted to migratory water birds from domestic poultry probably in China.

[Materials and methods] The swabs and the fecal materials were collected from domestic and feral birds in South Vietnam. Viral genes of influenza A virus were detected by RT-LAMP method. The RT-LAMP positive samples were inoculated to 10-day-old embryonated chicken eggs for virus isolation. The isolates were subtyped by HI and NI tests. Their genes were sequenced and phylogenetically analyzed. Pathogenicity of three H9 virus isolates were assessed in chickens, pigs, and mice.

[Results] Thirty-nine viruses were isolated from 183 viral gene positive materials in 858 swabs and fecal samples. Number and subtypes of the isolates were 26 H9N2, 1 H3N2, 1 H3N8, 7 H4N6, 3 H11N3, and 1 H11N9. All of the H9N2 isolates were classified into the Korean and G1 sublineage by phylogenetic analysis. Virus was not recovered from the lungs of chicken inoculated with Dk/VN/OIE-2327/09, Dk/VN/OIE-2328/09 and Dk/VN/OIE-2583/09 3 d.p.i, although antibody was detected from the chicken sera 14 d.p.i. Viruses were recovered from nasal wash of pig inoculated with these strains. Viruses were recovered from the lungs of mice inoculated with these strains and antibody was detected from mice sera 14 d.p.i. Body weight loss of mice inoculated with Dk/VN/OIE-2583/09 was decreased significantly.

[Discussion] These viruses of various subtypes have been isolated in the present study, demonstrating that influenza viruses are prevailing in poultry and wild birds in Vietnam. H9N2 viruses were replicated in pig, suggesting that H9N2 viruses have a possibility to acquire the ability for transmission to humans by passage in pig, and surveillance of swine influenza is important.

P-26 Studies on the pathogenicity of influenza virus in chicken

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Highly pathogenic avian influenza viruses (HPAIVs) cause lethal systemic infection in chickens. The cause of the death of the chickens infected with HPAIVs is poorly understood. We found that 50 % chicken lethal dose of A/turkey/Italy/4580/1999 (H7N1) (Ty/Italy/99) was 10^{-4} of that of A/chicken/Netherlands/2568/2003 (H7N7) (Ck/NL/03). In the present study, each of the strain was inoculated intranasally into four-week-old chickens and examined virologically and pathologically in order to throw light on the mechanisms how HPAIVs exert their pathogenesis.

Ty/Italy/99 replicated efficiently in each of the tissue including the brain and heart of chickens examined, causing sudden death within four days post-inoculation. On the other hand, Ck/NL/03 replicated more slowly than Ty/Italy/99 and killed chickens in six or seven days post-inoculation. The viruses in the blood of the chickens infected with Ty/Italy/99 rapidly increased and be maintained until they died, while these of viruses were detected in the blood of chickens infected with Ck/NL/03 transiently. It is suggested that efficiency of viral replication in the endothelial cells are different between these strains. In the histopathological analysis, more severe inflammatory lesions were found in the brains and hearts of the chickens infected with Ck/NL/03 than in those of the chickens infected with Ty/Italy/99. The present results indicate that the cause of the death of the chickens infected with Ty/Italy/99 may be different from that with Ck/NL/03, such as multiple organ disorder induced by cytokine storm.

Future studies are needed on viral replication in the endothelial cells and innate immune response to the infection with these viruses in order to determine the factors responsible for pathogenicity of influenza viruses in chickens.

P-27 Antigenic analysis of current H5N1 highly pathogenic avian influenza viruses and efficacy of an inactivated vaccine prepared from non-pathogenic virus isolates

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Outbreaks of highly pathogenic avian influenza (HPAI) caused by H5N1 viruses have become endemic at poultry farms in 63 countries. We have been conducting surveillance of avian influenza. Two genetically distinct H5N1 virus groups, clade 2.3.2 and clade 2.3.4 were isolated from wild birds in Asia recently. In the present study, we analyzed the antigenicity current isolated by hemagglutination-inhibition tests. Two HPAI viruses, A/whooper swan/Hokkaido/1/2008 (H5N1) and A/peregrine falcon/Hong Kong/810/2009 (H5N1) were classified into clade 2.3.2 and clade 2.3.4 respectively, were antigenically distinct each other and different from the isolates from fecal samples of wild birds. To assess the efficacy of an avian influenza vaccine prepared from non-pathogenic virus isolates against those viruses, A/duck/Hokkaido/Vac-3/2007 (H5N1) was propagated in embryonated chicken eggs, inactivated with formalin and adjuvanted with mineral oil. The Vac-3 vaccine was injected into the muscle of four-week-old chickens and they are challenged with A/whooper swan/Hokkaido/1/2008 (H5N1) or A/peregrine falcon/Hong Kong/810/2009 (H5N1) to assess the efficacy of the vaccine in clinical protection and reduction of virus shedding.

P-28 Genetic Characterization of Influenza A Viruses Isolated from Wild Waterfowl in Zambia

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Although the spread of highly pathogenic H5N1 avian influenza viruses (AIV) has stimulated surveillance programmes in wild birds and poultry worldwide, limited influenza virus surveillance in African waterbirds exist. We describe the genetic characterization of twelve AIV belonging to five distinct subtypes isolated from wild birds in Zambia from 2008-2009. Phylogenetic analyses demonstrated that AIV isolated in southern Africa formed distinct clusters within the Eurasian lineage, suggesting that there could be some degree of geographical segregation of host species that allows AIV to independently evolve in this region. Phylogenetic analyses also showed that some internal protein genes of the isolates from waterfowl in Zambia were closely related to those of influenza viruses isolated from wild birds, domestic ducks, and ostriches in South Africa, thus providing further evidence implicating wild birds in the introduction of AIV into South African farmed ducks and ostriches. Sequence analysis of the internal proteins revealed a high rate of human-type amino acids, suggesting that some of these strains may have the potential to directly infect humans. Our data indicates that some influenza virus strains that may have potential for enhanced interspecies transmission among avian and mammalian species could be maintained in some wild aquatic birds prevalent in southern Africa.

Abstract Poster Presentation

P-29 Predicting of the Antigenic Change of the Pandemic (H1N1) 2009 Influenza Virus Hemagglutinin

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Background: The pandemic influenza virus (2009 H1N1) was recently introduced into the human population. The hemagglutinin (HA) gene of 2009 H1N1 is derived from “classical swine H1N1” virus, which likely shares a common progenitor with the human H1N1 virus that caused the pandemic in 1918. Since antigenic changes of influenza virus HA occur more slowly in swine than in the human population, it was suggested that 2009 H1N1 might still retain an antigenic structure similar to that of 1918 H1N1 or the early isolates of its descendants. In this study, we compared HA antigenic structures of 2009 H1N1 and human H1N1 viruses by bioinformatics methods to investigate shared epitopes in known antigenic regions. We further discussed possible directions of antigenic changes in the evolutionary process of 2009 H1N1.

Materials and Methods: All the sequences used in this study were downloaded from the NCBI Influenza Virus Resource. First, we generated three-dimensional structures of the HA molecules of the 1918 pandemic, recent seasonal human H1N1 viruses, and 2009 H1N1 by using molecular modeling methods and compared their antigenic structures corresponding to known antigenic regions on the H1 HA. We then analyzed the number of *N*-glycosylation sequons and potential candidate codons that were not sequons, but able to become sequons with 1-3 nucleotide mutations (i.e., a set of three codons that required single, double, or triple nucleotide substitutions to produce sequons), in their globular head region of HAs.

Results: We found that HAs of 2009 H1N1 and the 1918 pandemic viruses shared a significant number of amino acid residues in known antigenic sites, suggesting the existence of common epitopes for neutralizing antibodies cross-reactive to both HAs. We thus hypothesize that the 2009 H1N1 HA antigenic sites involving the conserved amino acids will soon be targeted by antibody-mediated selection pressure in humans. Then we speculate that 2009 H1N1 will undergo similar patterns of amino acid substitutions in HA to those seen in seasonal human H1N1 viruses during its epidemic period. Indeed, such amino acid substitutions have been already found in the recent variants of the 2009 H1N1 virus.

While 2009 H1N1 HA lacks the multiple *N*-glycosylations that have been observed in the antigenic change of the human H1N1 virus during the early epidemic of this virus, 2009 H1N1 HA still retains unique three-codon motifs, some of which became *N*-glycosylation sites via a single nucleotide mutation in the human H1N1 virus. Therefore, this suggests the likelihood of additional *N*-glycosylation at these sites during future antigenic changes of 2009 H1N1 HA. Notably, some of the recent 2009 H1N1 variants have an additional *N*-glycosylation sequon at the same position where 1918 H1N1 virus readily acquired an *N*-glycosylation site during the circulation.

Conclusions: The present study suggests that the antigenic structure of 2009 H1N1 HA was still similar, at least in part, to that of the 1918 H1N1 HA. The 2009 and 1918 H1N1 HA shares unique three-codon motifs which are important to readily acquire *N*-glycosylation sequons in their globular head region. Based on these similarities, we predicted possible amino acid substitutions that might be associated with future antigenic change of 2009 H1N1, and confirmed that such substitutions occurred in some of the recent variants of this virus. Thus, the present study provides an insight into future likely antigenic changes in the evolutionary process of 2009 H1N1 in the human population.

P-30 Effect of IFN-alpha/beta signal on immune response against influenza virus infection

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The immune response can be divided into innate and adaptive components that synergise in the clearance of virus. Innate cytokine responses, such as production of interferon alpha/beta (IFN-alpha/beta), play some roles in regulating the antiviral pathway in the initial stages of infection and in shaping the inflammatory response and downstream of adaptive immune responses. Cytotoxic CD8 T cells play pivotal roles in virus elimination in the late stages of infection as adaptive immune responses. But previous studies on the effects of IFN-alpha/beta signal on protection from influenza virus infection have led to various conclusions. To clarify this, C57BL/6 wild-type (WT) and IFN-alpha/beta receptor deficient (IFNAR^{-/-}) mice were infected intranasally with influenza virus A/Fort Monmouth/1/47 (H1N1) strain. Then, we analyzed the effect of IFN-alpha/beta signal on the replication of influenza virus and adaptive immunity in the lungs of WT and IFNAR^{-/-} mice. We found that the virus titers in the lungs of IFNAR^{-/-} mice were significantly higher than those of WT mice on day 3 after infection. The lower IL-12p70 production was detected in the lungs of IFNAR^{-/-} mice on days 3 and 9 after infection as compared with that of WT mice. The percentage of IFN-gamma⁺ CD4⁺ T cells (Th1) in the all CD4⁺ T cells was significantly lower in the lungs on day 7 after infection in IFNAR^{-/-} mice as compared with WT mice. On day 7 after infection, the cytotoxicity of CD8 T cells in the lungs was significantly declined in IFNAR^{-/-} mice. These data demonstrate that IFN-alpha/beta signal is important not only for the elimination of virus in the initial stages of infection but also for differentiation of naïve CD4 T cells to Th1 cells and acquisition of effective cytotoxic function of CD8 T cells in the late stages of infection.

P-31 The role of antibody in heterosubtypic protective immunity against influenza virus infection (plan)

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Background

Influenza viruses are classified into types A, B, and C on the basis of the antigenic differences of their internal nucleoprotein and matrix protein. Influenza A viruses are further divided into subtypes based on the antigenic properties of the hemagglutinin (HA) and neuraminidase (NA) glycoproteins expressed on the surface of virus particles. Today, in Japan, inactivated split vaccines by subcutaneous injection are used for prevention of seasonal human influenza. But these vaccines hardly work against influenza viruses which belong to different subtypes from the vaccine strain. Even within the same subtype, the efficacy of influenza vaccine is highly dependent on the HA antigenicity of the epidemic strain.

Since 1997, human infections with highly pathogenic avian influenza virus have been reported one after another. Moreover, last year, swine-origin influenza virus infected humans and has spread around the world. Since it is difficult to predict the emergence of new pandemic influenza, we can not prepare suitable vaccines in advance. Thus, new strategies for prevention of influenza are needed.

Recently, it has been reported that intranasal immunization of mice induces a broad spectrum of heterosubtypic immunity against influenza virus infection. But the mechanism by which heterosubtypic protection occurs is poorly understood. Cytotoxic T lymphocyte (CTL), antibodies against common HA epitopes among subtypes, and intracellular or cell-surface neutralization by IgA antibody which acts mainly in mucosal immunity are hypothesized to be responsible for heterosubtypic protective immunity.

Objective

In this study, we will focus on anti-HA antibodies. We will investigate the class, quantity, and binding ability to multiple HA subtypes of antibodies induced after intranasal or subcutaneous inoculation of inactivated virus. And we are going to discuss about the role of anti-HA antibody in heterosubtypic protective immunity.

Methods

Mice will be immunized intranasally or subcutaneously with 100 μ g inactivated virus three times at three-week intervals. Seven days after the last inoculation, serum, nasal wash, and lung wash samples will be collected. By enzyme-linked immunosorbent assay (ELISA) and neutralization test, we will quantify anti-HA IgG and IgA antibodies that have ability of binding to multiple HA subtypes. Then, we will conduct experimental infection of immunized mice with influenza viruses of different HA subtypes, and investigate the heterosubtypic protective immunity.

Prospective result

Compared to subcutaneous inoculation, intranasal inoculation will induce antibodies which have ability to bind to HA of multiple subtypes. And the quantity and ability of anti-HA antibody may correspond to the heterosubtypic protective immunity.

P-32 Experimental study on antibody-mediated heterosubtypic immunity against influenza virus infection

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Influenza virus has two surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA), and 16 HA and 9 NA subtypes have been identified so far. HA is responsible for virus binding to host cell receptors, internalization of the virus, and subsequent membrane fusion. Neutralizing antibodies recognizing HA play a critical role in protection from influenza A virus infection. It is generally believed that the neutralizing antibodies to HA are not broadly cross-reactive among HA subtypes.

However, we succeeded in producing the monoclonal antibody (MAb) S139/1 which showed a broad reactivity to multiple HA subtypes in enzyme-linked immunosorbent assay, neutralization, and hemagglutination inhibition tests. To investigate the ability of MAb S139/1 to protect mice from influenza virus infection, mice were passively immunized with the antibody and challenged with A/WSN RG/33 (WSN) (H1N1), A/Adachi/2/57 (Adachi) (H2N2), and A/Aichi/2/68 (Aichi) (H3N2). In lungs of mice treated with MAb S139/1 one day before infection, WSN, Adachi, and Aichi titers were significantly lower than those of control mice. Treatment of mice three days after challenge still gave protective effects against lethal infection with WSN.

These results showed that passive immunization of MAb S139/1 had prophylactic and therapeutic efficacy in mice against multiple strains of different subtypes and neutralizing activity of MAb S139/1 *in vitro* was correlated with protective efficacy *in vivo*. While previously reported neutralizing MAbs that were cross-reactive to multiple HA subtypes are known to inhibit membrane fusion, MAb S139/1 inhibits receptor binding. This study demonstrates that antibodies inhibiting receptor binding also play an important role in heterosubtypic immunity against influenza virus infection.

Abstract Poster Presentation

P-33 Molecular characterization of immunoinhibitory factors PD-1/PD-L1 in bovine leukemia virus-infected cattle

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An immunoinhibitory receptor, programmed death-1 (PD-1), and its ligand, programmed death-ligand 1 (PD-L1), are involved in the immune evasion mechanisms for several pathogens causing chronic infections. The blockade of the PD-1/PD-L1 pathway by antibodies specific to either PD-1 or PD-L1 resulted in the re-activation of immune reactions, and is expected to be applied to new therapies for the chronic infectious diseases. However, few functional analyses of these molecules have been reported for domestic animals. Thus, in this study, cDNAs encoding for bovine PD-1 and PD-L1 were cloned and sequenced, and then, their expression and roles were analyzed in the bovine leukemia virus (BLV)-infected cattle.

Full length cDNA sequences encoding for bovine PD-1 and PD-L1 were cloned, and deduced amino acid sequences of bovine PD-1 and PD-L1 showed high homologies with those of human and mouse PD-1 and PD-L1, respectively. Functional domains, including immunoreceptor tyrosine-based inhibitory motifs in the intracellular domain of PD-1, were well conserved among cattle and other species.

PD-1 mRNA was predominantly expressed in T cells while PD-L1 mRNA was detected in monocyte-lineage cells. Both PD-1 and PD-L1 mRNAs were upregulated in peripheral blood mononuclear cells (PBMCs) by the stimulations with Concanavalin A or anti-CD3 antibody.

Next, the expression of PD-L1 was analyzed on PBMCs from BLV-infected cattle at the asymptomatic (AL), persistent lymphocytosis (PL), and enzootic bovine leukemia (EBL) stages by flow cytometry. The PD-L1 expression on PBMC especially B cells in cattle at the PL and EBL stages were higher than that in cattle at the AL stage or uninfected cattle. The level of the PD-L1 expression was correlated with the degree of the disease progression. The blockade of the PD-1/PD-L1 pathway in PBMC by either PD-L1-specific antibodies or recombinant PD-L1 upregulated the productions of IL-2 and IFN- γ in both BLV-infected and uninfected cattle, as well as inhibited the proliferation of BLV in PBMC from BLV-infected cattle.

The immunoinhibitory functions of bovine PD-1 and PD-L1 were shown in this study, and the PD-L1-induced immunoinhibition may play one of the key roles on the disease progression of the chronic infection with BLV. Thus, immune re-activation, such as the blockade of the PD-1/PD-L1 pathway, would be essential to protect cattle from BLV infection and to inhibit disease progression.

P-34 Amino acid substitutions or insertion in the Meq proteins could affect their transactivation and transformation abilities

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Marek's disease virus (MDV), known as one of the most oncogenic herpesviruses, causes T cell lymphoma in its natural host, chicken. Marek's disease (MD) has been controlled by the administration of live vaccines of nonpathogenic strains of MDV. However, recent field isolates of MDV tend to increase their virulence, and the risk of future outbreaks is pointed out. The diversity or insertion in amino acid residues of Meq, an oncoprotein of MDV, among a variety of MDV strains has been reported. There are distinct diversity and point mutations present in Meq of highly virulent MDV strains, whereas L-Meq, containing 60 amino acid insertion in the transactivation domain of Meq, is identified in low virulent MDV strains. Since Meq or L-Meq could regulate the expressions of viral and cellular genes as a transcriptional factor, these findings suggest that the diversity or insertion in Meq may correlate with the difference in the transactivation activities of Meq or L-Meq, and is considered as an important factor related to the virulence. In this study, we analyzed the relationship between the diversity or insertion and the transactivation activities of Meq by reporter assay. The transactivation activities of Meq were altered when the amino acid substitutions were introduced into the transactivation domain, whereas the transactivation activity of L-Meq was significantly weaker than that of Meq. These results indicate that the diversity or insertion present in the Meq proteins could affect their transactivation activities. In addition, evaluation of transforming potentials based on anchorage-independent cell growth using the DF-1 chicken embryo fibroblast transformation system showed the significantly lower number of colony formation in L-Meq- than Meq-expressing DF-1 subclones. These results suggest that the tumorigenesis of L-Meq is weaker than that of Meq, and therefore, MDV strains encoding L-Meq is low virulent. In addition, we analyzed the correlation between the virulence and amino acid substitutions in basic region 2 (BR2), because BR2 is important for the nucleolar localization and DNA binding of Meq. The amino acid substitutions in BR2 resulted in the change of the transactivation and transformation activities. Therefore, the diversity in BR2 could also affect the function of Meq. Thus, the diversity or insertion present in the Meq proteins could affect their transactivation and transformation activities and might be a determinant of the virulence of MDV strains.

P-35 Prevalence and Carrier Status of Leptospirosis in Smallholder Dairy Cattle and Peridomestic Rodents in Kandy, Sri Lanka

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Introduction

Leptospirosis is an important bacterial zoonotic disease globally and one of the notifiable diseases in Sri Lanka. Other than human leptospirosis, little information is available on leptospirosis in domestic and feral animals in Sri Lanka. Thus, this study attempted to determine the prevalence and carrier status of leptospirosis in smallholder dairy cattle and peridomestic rodents to understand the impact on public health in Kandy, Sri Lanka.

Materials and Methods

Cattle and rodent samples were collected from the Yatinuwara and Udunuwara divisional secretaries in Kandy. Serum samples were analyzed for the presence of anti-leptospiral antibodies using microscopic agglutination test. DNA was extracted from cattle urine and rodent kidney tissue samples, in which polymerase chain reaction was carried out to detect the *Leptospira* flaB gene.

Results

Out of 113 cattle serum samples, 23 (20.3%) were positive; 16 (69.5%) and 6 (26%) reacted with serogroups Sejroe and Hebdomadis, respectively. Of the 74 rodent samples, thirteen (17.5%) were positive; eight and four had reactions to serogroups Javanica and Icterohaemorrhagiae, respectively. Leptospiral DNA was detected in one cattle urine sample and was identified as *Leptospira interrogans*. The similar *Leptospira* serogroups were detected in cattle and rodents from the same area.

Conclusion

This study revealed a high prevalence of leptospirosis in cattle and rodents in Kandy, Sri Lanka. These animals were infected with a wide array of leptospiral serogroups. These results suggest that these animals may act as reservoirs of leptospirosis in the area and pose a potential risk to agricultural community in Kandy, Sri Lanka.

P-36 Behavioural pattern of rural residents of Kandy, Sri Lanka in relation to leptospirosis: implications for intervention

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Introduction

Leptospirosis is a zoonotic disease that has emerged as an important cause of morbidity and mortality among impoverished populations, such as those in the rural communities in Kandy, Sri Lanka but poor knowledge of the disease may be associated with the increasing incidence. To inform future interventions, knowledge, attitudes and practices (KAP) on leptospirosis among rural residents of Kandy, Sri Lanka were assessed.

Methods

An interview-based survey involving 159 rural residents of Kandy in the Central Province of Sri Lanka was carried out from September to October 2009. The KAP variables along with socio-demographic information were measured using the yes/no question format. Knowledge responses were first recoded and constructed into 'low' and 'high' level categories.

Results

Respondents were of mean age (mean=44.28 years; SD±15.35); mostly men and married; of differing educational levels; primarily farmers; of low-income category; and were residing in the rural community for diverse periods. Most of the respondents have good attitudes and practices towards leptospirosis. In 4 of the 5 knowledge items analyzed, data revealed that there were far greater numbers of respondents with low than high knowledge level of leptospirosis. Further cross tabulation analysis between income level and knowledge items revealed a statistically significant association, especially in knowledge of symptoms. Respondents with high income appeared to have higher knowledge.

Conclusion

Rural residents of Kandy across socio-demographic categories are at a continuing risk for leptospirosis because of their poor knowledge. Appropriate interventions are needed.

Abstract Poster Presentation

P-37 Functional analysis of an α -helical antimicrobial peptide derived from a novel mouse defensin-like gene

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Gene-encoded antimicrobial peptides (AMPs) are an essential component of the innate immune system in many species. Analysis of β -defensin gene expression in mouse tissue using primers that were specific for conserved sequences located outside of the β -defensin translated region identified a novel small gene. The novel gene had an open reading frame of 114 basepairs and encoded a predicted protein of 37 amino acid residues. A search of the genome database revealed that the gene locus and the sequence of exon 1 of this novel gene were similar to subgroup 1 mouse β -defensins. A small peptide, K17 (FSPQMLQDIIEKKTIL), derived from the amino acid sequence of this novel gene was synthesized. K17 which possesses positive charge is predicted to assume an α -helical conformation by 3D conformation prediction database. Moreover, circular dichroism (CD) spectroscopy analysis of K17 demonstrates that K17 presents random coil conformation in aqueous solution, but adopts α -helical conformation in the presence of 50% trifluoroethanol, a membrane mimicking environment. The short, cationic and helical peptide is known to act as antimicrobial peptide. K17 exhibited bactericidal activity against *Salmonella enterica* serovar Typhimurium (Gram negative) and *Staphylococcus aureus* (Gram positive), but it was not cytotoxic in cultures of mammalian cells or hemolytic in cultures of erythrocytes. These results suggested that K17 may be a candidate therapeutic for the treatment of bacterial infection.

