The 3rd International

Young Researcher Seminar

for Zoonosis Control, 2011

September 16 (Fri)- 17 (Sat), 2011

Graduate School of Veterinary Medicine,Hokkaido University

As of Sept. 2, 2011

HOKKAIDO UNIVERSITY FOUNDED 1876

COVERING PHOTOS

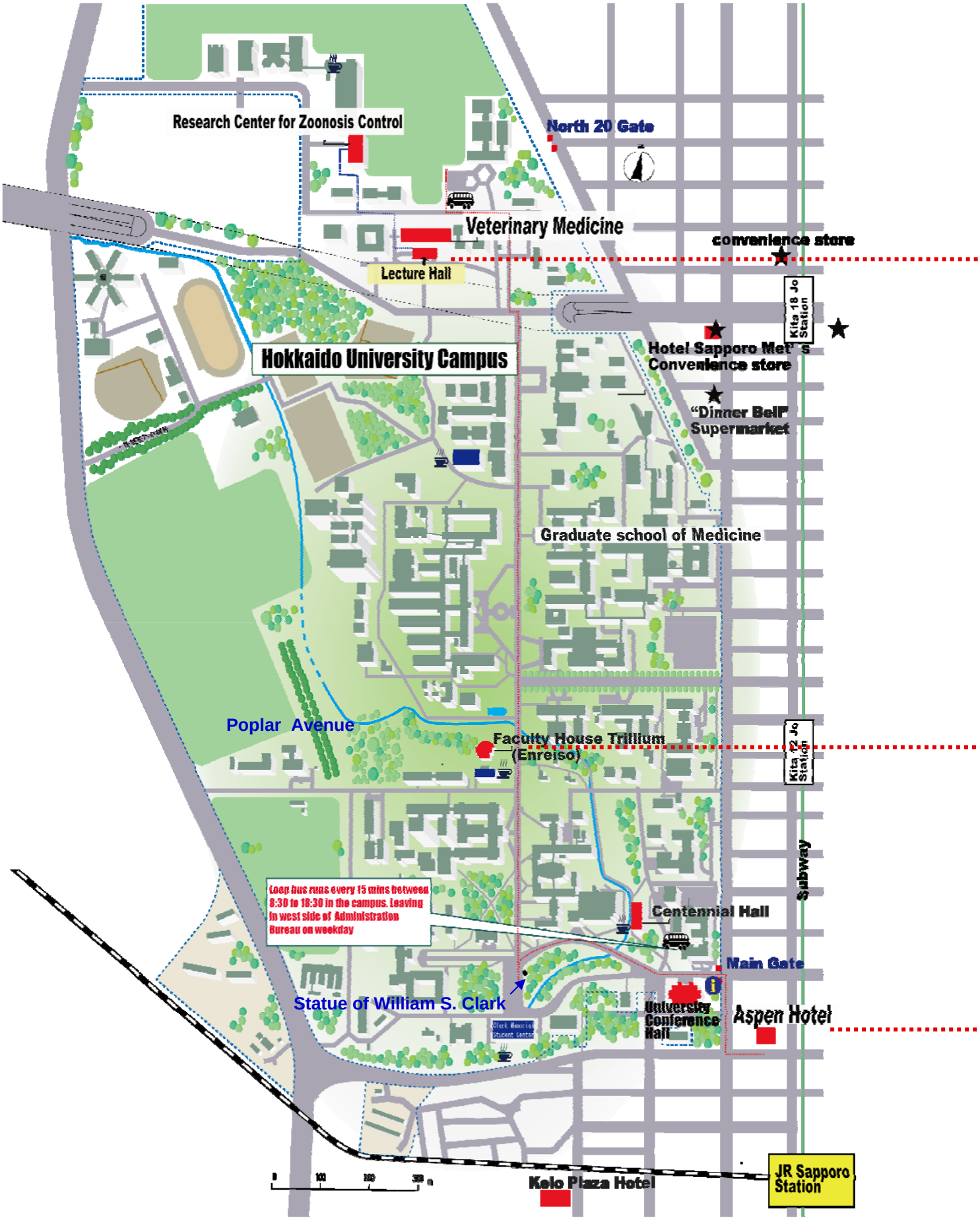
A. STATUE OF WILLIAM S. CLARK AT HOKKAIDO UNIVERSITY
William S. Clark came from Massachusetts to become Vice President of the germinal university in its founding year from August 1876 to April 1877. He helped introduce farming techniques suitable to Hokkaido conditions, and broadened students' minds to the outside world and to the spiritual sense of Christianity. The present statue erected in 1948 was modeled on the original which was melted down for metal during World War II.

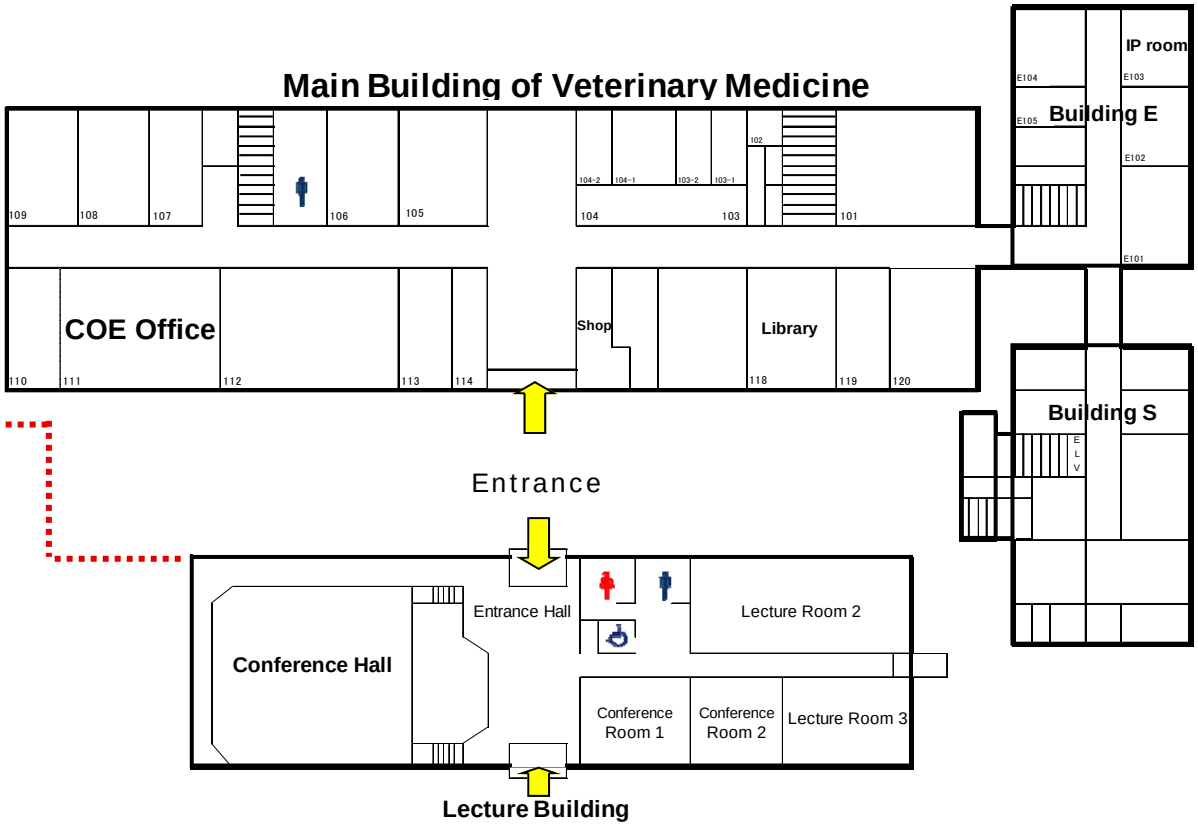
B. THE LECTURE BUILDING OF VETERINARY MEDICINE, HOKKAIDO UNIVERSITY
Venue of the seminar , constructed in April, 2010

C. POPLAR AVENUE
Poplars were first planted in Hokkaido in mid-Meiji as a wind break. Many of these are far past their normal lifespan of sixty years, and despite the danger of them crashing down, they are an essential part of the Sapporo tourist's itinerary.

CONTENTS

Venue:


Research Center for Zoonosis Control
North 20 Gate
Veterinary Medicine
Lecture Hall
convenience store
Kita 18 Jo Station
Hokkaido University Campus
Hotel Sapporo Met's
Convenience store
"Dinner Bell"
Supermarket
Graduate school of Medicine
Poplar Avenue
Faculty House Trillium
(Enreiso)
Kita 12 Jo Station
Subway
Loop bus runs every 15 mins between 8:30 to 18:30 in the campus. Leaving in west side of Administration Bureau on weekday
Centennial Hall
Main Gate
Statue of William S. Clark
University Conference Hall
Aspen Hotel
Kelo Plaza Hotel
JR Sapporo Station



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:	**Farewell party on Sept. 17**

Restaurant "Enreiso" at Faculty House Trillium:
Welcome reception from 18:30 to 20:30 on Sept. 16

Aspen Hotel:

Accommodation for the invited speakers
Address/ 5, Kita8-jyo Nishi4-chome, Kita-ku, Sapporo,
Phone +81 11-700-2111
FAX +81 11-700-2002
http://www.aspen-hotel.co.jp/english/frame.htm

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 8:15 to 9:15 on September 16 (Fri). You will have a programme & abstract, name card and information.

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 for Oral presentation

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. 16.

【Keynote lectures 】

Keynote presentation for Dr. Monique Lafon and Dr. Kisaburo Nagamune is allocated 60 min including discussion.
(Dr. Lafon and Dr. Nagamune do not need to prepare poster presentation)

【10 Invited speakers (Oral presentation)】

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

【12 speakers from Hokkaido University】

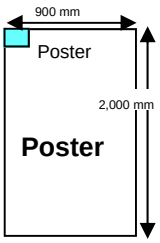
Oral presentation for 12 speakers from Hokkaido University is allocated 15 min including discussion.

Note: All participants (including invited and oral presenters from Hokkaido University except Keynote presenters) should prepare poster.
However, invited and oral presenters from Hokkaido University do not need prepare "One minute presentation (flash talk)".

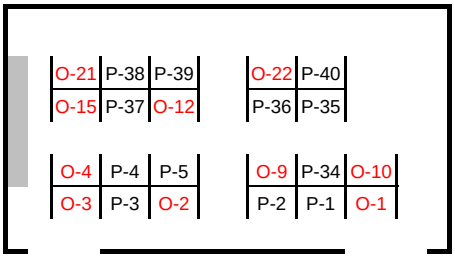
General Guideline for Poster Presentation

Poster Presentation

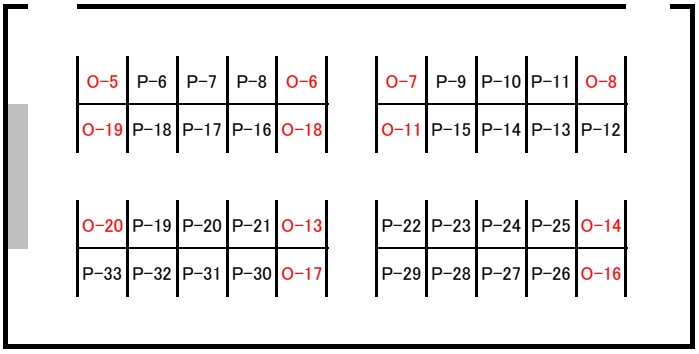
- Each board size is 900 mm W x 2,000 mm H
- A0 size: 841 mm x 1189 mm may be appropriate if you prepare your poster as a single sheet.
- Boards with Poster No. card (147 mm W x 105 mm H) and pushpins for poster setup will be provided.
- Each poster will be provided a poster number as indicated in the Programme & Abstract booklet.
- Poster is to be mounted from 8:15 to 9:15 in the morning of Sept. 16 at Poster presentation Venues as below.
- Poster is to be removed after the seminar on September 17.



Poster Presentation Venue: Lecture Room 3



Poster Presentation Venue: Lecture Room 2



One minute presentation (flash talk) by 40 poster presenters from Hokkaido University

Flash talk will be held on September 16 (9:35-10:30) at Conference Hall .
The presentation should be within one minute. Please prepare one ppt slide by September 9 and send it to GCOE office by E-mail.

【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

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)

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)

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, Dept. of Applied Veterinary Sciences, 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
Hokkaido University
Kita-18 Nishi-9 Kitaku, Sapporo, Japan 060-0818
tel. 011-706-5294

gcoe@vetmed.hokudai.ac.jp
http://www.vetmed.hokudai.ac.jp/gcoe/

FYI
Sapporo city
http://www.welcome.city.sapporo.jp/english/index.html
Sapporo Visitor Attractions
http://www.congre.co.jp/iums2011sapporo/data/accom.html
Planners Guide for Sapporo
http://www.conventionsapporo.jp/planners/e/
Narita International Airport (Tokyo)
http://www.narita-airport.jp/en/index.html
New Chitose Airport (Sapporo)
http://new-chitose-airport.jp/en/
JR (timetable New chitose airport—Sapporo)
http://www2.jrhokkaido.co.jp/global/english/ttable/04.pd
Hokkaido University Campus
http://www.hokudai.ac.jp/en/pickup/accesstocampus.html

Program Day 1

September 16 (fri), 2011

8:15 ~ 9:15		Registration, Submitting presentation data and setting up poster
9:20 ~ 9:35		Opening speech (Hiroshi Kida, Leader of Global COE Program, Professor, Hokkaido Univ.) Orientation (Yuji Sunden, Organizing Committee)
9:35 ~ 10:30	**Flash talk**	
10:30 ~ 10:45	++ Break ++	
10:45 ~ 12:00	**Oral presentation I** (Chair persons: Ryo Nakao & Jung-Ho Youn)	
	O-1	The role of cholesterol in C. burnetii pathogenesis Stacey D. Gilk (USA)
	O-2	Microbial manipulation of host transcriptional programming by nuclear toxins – the *Anaplasma phagocytophilum* AnkA model Sara H. Gilmore (USA)
	O-3	Impact of the E540V Amino Acid Substitution in GyrB of *Mycobacterium tuberculosis* on Quinolone Resistance Hyun Kim (Hokkaido Univ.)
	O-4	Seroprevalence of anti-leptospira antibodies among patients with acute febrile illness with renal dysfunction in spite of negative result with several laboratorial leptospira tests in Thailand Rie Isozumi (Hokkaido Univ.)
12:00 ~ 12:30	Lunch	
12:30 ~ 14:00	Zoonosis Control Center (CZC) tour for invited speakers	
14:00 ~ 15:00	**Keynote Lecture I** (Chair persons: Tatsuya Sakurai & Yusuke Sakai)	
		Apicomplexan parasites and plant hormones Kisaburo Nagamune (Japan)
15:00 ~ 15:15	++ Break ++	
15:15 ~ 16:30	**Oral presentation II** (Chair persons: Kyoko Hayashida & Jesca Nakayima)	
	O-5	Home renovating: how malaria parasites export remodelling proteins into parasitized human erythrocytes Justin A. Boddey (Australia)
	O-6	Regulatory dendritic cells expand and dampen pathogenicity during *Trypanosoma evansi* infection Hirohisa Mekata (Hokkaido Univ.)
	O-7	$CD4^{+}Foxp3^{+}$Regulatory T cells in hepatic *Leishmania donovani* persistence Saruda Tiwananthagorn (Hokkaido Univ.)
	O-8	Biological characterization of molecules from poultry red mite, *Dermanyssus gallinae* for the development of a new control method of the mite Masayoshi Isezaki (Hokkaido Univ.)
16:30 ~ 17:30	Poster core time I (Odd numbers and oral presentations at day 1) at Lecture room 2 & 3	
18:30 ~ 20:30	Welcome reception at Enreiso (Faculty House)	

Program Day 2

September 17 (sat), 2011

8:45 ~ 9:45 **Keynote Lecture II** (Chair persons: Yuji Sunden & Kenta Shimizu)

Evasive strategies of rabies virus
Monique Lafon (France)

9:45 ~ 9:55 ++ Break ++

9:55 ~ 11:30 **Oral presentation III** (Chair persons: Yukiko Sassa & Yosuke Nakayama)

O-9 E2 Antibody inhibits nonstructural protein processing and blocks intracellular Alphavirus replication
Kimberly L W Schultz (USA)

O-10 Non-neutralizing antibodies protect against lethal West Nile virus infection via Fc-gamma receptor and complement–dependent mechanisms
Matthew R. Vogt (USA)

O-11 The K627E amino acid substitution of the PB2 of A/Hong Kong/483/1997 (H5N1) influenza virus alters the efficiency of RNA synthesis of NP gene
Naoki Yamamoto (Hokkaido Univ.)

O-12 A single amino acid residue in major histocompatibility complex class I is a determinant for its function as an equine herpesvirus-1 receptor
Michihito Sasaki (Hokkaido Univ.)

O-13 Detection of newly generated PrP^{Sc} in Neuro2a cells inoculated with fluorescent-dye labeled purified PrP^{Sc}
Takeshi Yamasaki (Hokkaido Univ.)

11:30 ~ 13:00 Group Photo at the entrance of the Lecture Building ++ Lunch ++

13:00 ~ 14:20 **Oral presentation IV** (Chair persons: Shumpei P. Yasuda & Saya Kuribayashi)

O-14 Hantavirus infection in the Southeast of Brazil
Glauciane Garcia de Figueiredo (Brazil)

O-15 Investigation and phylogenetic analysis of novel arenaviruses in Zambia
Akihiro Ishii (Hokkaido Univ.)

O-16 Modelling the influence of habitat hot spots on the disease spread and vaccination strategies
Julio Benavides (France)

O-17 Filovirus-specific antibodies detected in fruit bats and nonhuman primates in Zambia
Eri Nakayama (Hokkaido Univ.)

14:20 ~ 14:30 ++ Break ++

14:30 ~ 16:10 **Oral presentation V** (Chair persons: Manabu Igarashi & Yayoi Otsuka)

O-18 H5N1 virus in Russia (2005-2011): molecular epidemiology, ecology and evolution.
Kirill Sharshov (Russia)

O-19 Reduction of sampling bias in large datasets by systematic trimming of phylogenetic trees
Kouki Yonezawa (Hokkaido Univ.)

O-20 Unlocking clues to influenza species specificity, virus transmissibility, and antiviral evasion through computational investigations of neuraminidase
Rommie E. Amaro (USA)

O-21 Single particle reconstruction and assembly of Rift Valley fever virus
Alexander Freiberg (USA)

O-22 Evaluation of the policies in the foot-and-mouth disease outbreak in Japan, 2010, and assessment of future outbreak risks
Norikazu Isoda (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 ~ 20:30 Award & Closing Speech (Chihiro Sugimoto, Professor, Hokkaido Univ.)
Farewell Party at Conference room

Profile of Invited speakers

Keynote lecture I

Kisaburo Nagamune

Chief
Division of Protozoology
Department of Parasitology
National Institute of Infectious Diseases
Japan
nagamune@nih.go.jp

ACADEMIC DEGREES:

D.V.M. 1992 Osaka Prefectural University (Veterinary Medicine)
Ph.D. 1996 Osaka University (Medical Science)

PROFESSIONAL APPOINTMENTS:

1996 - 1999 Postdoctoral Fellow, Research Institute for Microbial Diseases, Osaka University
1999 - 2008 Assistant Professor, Research Institute for Microbial Diseases, Osaka University
2004 - 2007 Postdoctoral Research Scholar, Department of Microbiology, Washington University in St. Louis
2008 - 2009 Assistant Professor, Graduate School of Life and Environmental Sciences, University of Tsukuba
2009 - 2010 Senior Research Scientist, Department of Parasitology, National Institute of Infectious Diseases
2010 - Chief, Department of Parasitology, National Institute of Infectious Diseases

RESEARCH INTERESTS:

Molecular and cell biology of parasites, especially *Toxoplasma gondii*.

Keynote lecture II

Monique Lafon

Head of Laboratory
Department of Virology
Laboratory of Viral NeuroImmunology
Institut Pasteur, Paris, France
Monique.lafon@pasteur.fr

ACADEMIC DEGREES:

1987	Doctorat d'Etat Biochimie/Microbiologie Université Paris VII
1982	Thèse de Doctorat de Troisième cycle Génétique Université d'Orsay
1978	Agrégation de Sciences Naturelles Option Sciences Biologiques
1975-1979	Ecole Normale Supérieure ENS Fontenay-St Cloud

PROFESSIONAL APPOINTMENTS:

2001-	Head of Viral NeuroImmunology laboratory
1994-	Associate Professor (chef de laboratoire) Institut Pasteur
1989-93	Assistant Professor (chargé de recherche) Institut Pasteur
1988-94	Deputy Director with Pr P Sureau Director WHO International Reference Center for Rabies
1983-88	Junior Investigator (assistant) Institut Pasteur
1980-82	Associate Scientist at the Wistar Institute, Philadelphia, USA

RESEARCH INTERESTS:

Rabies virus, West Nile Virus and Herpes simplex virus, neuron-virus interaction, immunoevasive strategies of viruses, innate immune response, apoptosis, neurosurvival.

Profile of Invited speakers

1

Stacey D. Gilk

Postdoctoral Fellow
Laboratory of Intracellular Parasites
Rocky Mountain Laboratories
National Institutes of Health, Hamilton, MT
gilks@niaid.nih.gov

ACADEMIC DEGREES:

B.S. 1998 University of Notre Dame (Biology)
Ph.D. 2004 University of Vermont (Microbiology and Molecular Genetics)

PROFESSIONAL APPOINTMENTS:

2004-2007 Postdoctoral Associate, University of North Carolina, Chapel Hill, NC
2007- Postdoctoral Fellow, Rocky Mountain Laboratories (NIH), Hamilton, MT

RESEARCH INTERESTS:

The primary focus of my research is to identify and characterize the mechanisms intracellular pathogens utilize to manipulate host cell lipids, using a combination of cell biology, molecular biology, and biochemical approaches. I am currently studying the intracellular bacterial pathogen *Coxiella burnetii,* a unique organism that thrives in the acidic environment of a modified phagolysosome, or parasitophorous vacuole. Specifically, I am interested in the lipids and lipid signaling pathways involved in the biogenesis and maintenance of the *C. burnetii* parasitophorous vacuole, and the role(s) of two unique bacterial sterol reductases in this process.

Sara Hideko Gilmore

PhD Candidate
Division of Pathology,
Department of Cellular and Molecular Medicine
School of Medicine, Johns Hopkins University
gilm5447@gmail.com

ACADEMIC DEGREES:

B.S.	2007	University of Idaho (Molecular Biology and Biochemistry)
M.S.	2009	Idaho State University (Pharmacology and Pharmaceutical Sciences)
Ph.D.	-	Johns Hopkins University, School of Medicine (Cellular and Molecular Medicine)

RESEARCH INTERESTS:

Host pathogen interactions are remarkably complex and have become increasingly intricate. Many pathogens are now widely recognized as agents that can actively alter transcriptional profiles in order to elicit a more hospitable environment. *Anaplasma phagocytophilum* is the causative agent of human granulocytic anaplasmosis and is transmitted by *Ixodes* ticks. *A. phagocytophilum* is an obligate intracellular bacterium of neutrophils that secretes the nuclear toxin AnkA, which appears essential for pathogenesis of disease. AnkA accumulates in the nucleus of infected cells over the course of infection and is known to bind DNA at AT rich regions. The laboratory is interested in how AnkA exerts its effects, particularly by altering the transcriptional profile of the host cell through epigenetic alterations.

Profile of Invited speakers

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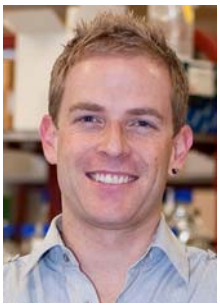

Justin Andrew Boddey

ARC QEII Fellow
Division of Infection and Immunity
The Walter and Eliza Hall Institute of Medical Research
Melbourne, Australia
boddey@wehi.EDU.AU

ACADEMIC DEGREES:

B.Biomed.Sci	2000	Griffith University
B.Biomed.Sci (Hons I)	2001	Griffith University
Ph.D.	2006	Griffith University

PROFESSIONAL APPOINTMENTS:

2011	Australian Research Council QEII Fellow
2010	Senior Postdoctoral Fellow, Walter and Eliza Hall Institute
2006 - 2009	Postdoctoral Fellow, Walter and Eliza Hall Institute

RESEARCH INTERESTS:

Molecular cell biology and pathogen-host interactions. My research investigates the exploitation of host cells by intracellular pathogens and is aimed at understanding these processes at the molecular level. This involves genetics, biochemistry, molecular biology and proteomics to study protein trafficking. I have worked on *Burkholderia pseudomallei* and now focus on the malaria parasite, predominantly *Plasmodium falciparum*.

Kimberly Schultz

Postdoctoral Fellow
Department of Molecular Microbiology and Immunology
Bloomberg School of Public Health
Johns Hopkins University
kischult@jhsph.edu

ACADEMIC DEGREES:

B.S.	2002	State University of New York at Geneseo College (Biology)
Ph.D.	2008	University of Wisconsin – Madison (Cellular and Molecular Biology)

PROFESSIONAL APPOINTMENTS:

2008 – present	Postdoctoral Fellow (Johns Hopkins Bloomberg School of Public Health)

RESEARCH INTERESTS:

Alphaviruses are transmitted by mosquitoes and cause encephalitis in mammals. Due to the ease of spread and high mortality rates, alphaviruses have been developed as biowarfare agents. We study Sindbis virus, which causes encephalitis in mice. Regulating viral infections in the central nervous system poses a unique challenge to the immune system. Clearance must occur without damaging neurons, which are nonrenewable. My work focuses on understanding the role of antibody in control of alphavirus infections. Ultimately, determination of the molecular mechanism by which antibody restricts intracellular alphavirus replication will identify potential antiviral targets.

Profile of Invited speakers

5

Matthew R. Vogt

M.D./Ph.D. Student
Medical Scientist Training Program
Immunology Program
Washington University School of Medicine, St. Louis, U.S.A.
mrvogt@wustl.edu

ACADEMIC DEGREES:
B.A. 2005 Washington University in St. Louis (Biology)

RESEARCH INTERESTS:
My general research interests are at the crossroads of virology and immunology. Specifically, my graduate work has been focused on studying mechanisms of neutralization and protection provided by antibodies against West Nile virus.

6

Glauciane Garcia de Figueiredo

Ph.D. Student
Virology Research Center, School of Medicine of
the University of São Paulo in Ribeirão Preto, Brazil
glaucianegf@yahoo.com.br

ACADEMIC DEGREES:

B.A.	2005	Federal University of Sao Carlos- São Carlos- Brazil (Biological Science)
M.A.	2009	School of Medicine of the University of São Paulo in Ribeirão Preto, Brazil (Microbiology in Applied Imunology)
Ph.D.Student	2009	Medical Clinical Department of School of Medicine of the University of São Paulo in Ribeirão Preto, Brazil (Biomedical Science)

RESEARCH INTERESTS:
Emerging Zoonoses, mainly Hantavirus and Arenavirus

Profile of Invited speakers

7

Julio Benavides

Ph.D candidate
Institute of Evolutionary Sciences of Montpellier
CNRS-University of Montpellier II
Ph.D student affiliated to Institute of Zoology, ZSL
benavidesjulio@yahoo.fr

ACADEMIC DEGREES:

B.Sc	2006	University of Montpellier II	(Population Biology)
M.Sc	2008	University of Montpellier II	(Evolutionary biology and Ecology)
Ph.D	2008-Present	University of Montpellier II	(Wildlife epidemiology)

PROFESSIONAL APPOINTMENTS:

2009 - 2010 Principal Investigator: Impact of human activities in the dynamics of *E. coli* in western lowland gorillas (*Gorilla gorilla gorilla*). Dzanga-Ndoki National Park, Central African Republic and Lope National Park, Gabon.

2008 Member of the "Efficient Wildlife Disease Control: From Social Network Self-organization to Optimal Vaccination" working group, at the National Center for Ecological Analysis and Synthesis, University of California Santa Barbara, USA. Group leader: Peter Walsh.

RESEARCH INTERESTS:

My research interests encompass the general processes of evolutionary biology and population dynamics, but more particularly disease ecology. Throughout my Ph.D I have focused on understanding how environmental factors such as habitat heterogeneity and animal density can impact parasite transmission in socially structured populations. I address these questions by developing agent-based models and also by collecting samples with non-invasive techniques in the field. As such, my field work focuses on the transmission of antibiotic resistance from humans to wild animals, including western lowland gorillas.

8

Kirill Sharshov

PhD
Head of Laboratory of Influenza Virus Ecology,
Zoonotic Infections and Influenza Department,
State Research Center of Virology and Biotechnology “Vector”,
Novosibirsk, Russia

Researcher
Novosibirsk State University,
Novosibirsk, Russia
sharshov@yandex.ru

ACADEMIC DEGREES:

M Sci. 2005	Tomsk State University, Russia (Biology)
Ph.D. 2010	State Research Centre of Virology and Biotechnology “Vector”, (Virology)

PROFESSIONAL APPOINTMENTS:

2005 - 2007	Junior researcher of Zoonotic Infections and Influenza Department, State Research Centre of Virology and Biotechnology “Vector”
2007 - 2010	Researcher of Zoonotic Infections and Influenza Department, State Research Centre of Virology and Biotechnology “Vector”
2010 - present	Head of Laboratory of Influenza Virus Ecology in Zoonotic Infections and Influenza Department, State Research Centre of Virology and Biotechnology “Vector”, Novosibirsk, Russia; Researcher of Novosibirsk State University, Novosibirsk, Russia

RESEARCH INTERESTS:

The research focuses primarily on the Molecular virology and Molecular epidemiology of Avian Influenza viruses, Ecology of Influenza viruses and their hosts, mechanisms for interspecies transmission, studying of Highly pathogenic avian influenza (HPAI) viruses and their pathogenicity using animal models, antigenic variability of influenza virus, Influenza virus evolution. Also of interest are the Molecular epidemiology and Virology of Newcastle Disease Virus (NDV) circulating in wild bird populations and poultry.

9

Rommie E. Amaro

Assistant Professor
Departments of Chemistry, Computer Science, and Pharmaceutical Sciences
College of Health Sciences
University of California, Irvine
ramaro@uci.edu

ACADEMIC DEGREES:

B.S.	1999	University of Illinois at Urbana-Champaign (Chemical Engineering)
Ph.D.	2005	University of Illinois at Urbana-Champaign (Chemistry)

PROFESSIONAL APPOINTMENTS:

2000 - 2001	Associate Research Engineer, Kraft Foods, Inc.
2005 - 2009	NIH Postdoctoral Fellow, University of California, San Diego
2009 -	Assistant Professor, University of California, Irvine

RESEARCH INTERESTS:

The Amaro lab is broadly concerned with the development and application of state-of-the-art computational and theoretical techniques to investigate the structure, function, and dynamics of complex biological systems. At the interface of chemistry, biology, physics, and pharmacology, our research integrates both applied and basic science components, with goals to bridge the interface between basic and clinical research. Fundamental enzymological and drug discovery studies are tightly coupled to a wide range of biochemical and biophysical experiments that allow us to engage in dynamic and exciting collaborations with various experimental labs.

Alexander Freiberg

Assistant Professor
Director Robert E. Shope BSL-4 Laboratory
Department of Pathology
University of Texas Medical Branch
Galveston, Texas 77550, USA
anfreibe@UTMB.EDU

ACADEMIC DEGREES:

Diploma	2000	University of Potsdam, Germany (Biochemistry)
Ph.D.	2005	University of Potsdam, Germany (Physical Biochemistry)

PROFESSIONAL APPOINTMENTS:

1996-2000	Research Assistant/Student, Institute for Biochemistry and Biology, University of Potsdam, Germany
2001	Visiting Scientist, Haematology, Oncology and Tumorimmunology, Max-Delbrück-Center for Molecular Medicine, Berlin, Germany
2001-2005	Ph.D. Candidate, Institute for Biochemistry and Biology, University of Potsdam, Germany
2005-2009	Postdoctoral Fellow, Department of Pathology, University of Texas Medical Branch (UTMB), Galveston, USA
2009-present	Assistant Professor, Department of Pathology, UTMB, Texas
2009-present	Director, Robert E. Shope BSL-4 Laboratory, UTMB, Texas
2010-present	Member of the Sealy Center for Vaccine Development, UTMB, Texas
2010-present	Member of the Center for Biodefense and Emerging Infectious Diseases, UTMB, Texas
2010-present	Member of the Institute for Human Infections and Immunity, UTMB, Texas
2011-present	Member of the Center for Tropical Diseases, UTMB, Texas

RESEARCH INTERESTS:

My laboratory is interested in viral pathogenesis, virus assembly, and vaccine and antiviral drug development.

Keynote Lecture I (Day 1: 14:00-15:00 on September 16)

Keynote lecture I

Apicomplexan parasites and plant hormones

Kisaburo Nagamune
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Recently, we found that *Toxoplasma gondii* produces a plant hormone, abscisic acid, and that it is essential for the infection of this parasite. So, we studied the effect of other plant hormones cytokinins on *Toxoplasma gondii*. We found that genes encoding the enzymes for the *trans*-zeatin (tZ) biosynthesis in plants are present in *T. gondii* genome. Consistently, we detected intermediates for two possible tZ-biosynthesis pathways by MS analysis. A natural cytokinin, tZ, accelerated the proliferation of *T. gondii*. In contrast, a synthetic cytokinin, thidiazuron, inhibited the parasite proliferation in culture. This inhibition was also observed in laboratory animals. Since cytokinins regulate the cell cycle progression by enhancing the expression of D-type cyclin in plant, we carried out flowcytometric analysis to observe the effect of cytokinins on the cell cycle progression of *T. gondii*. TZ hastened the cell cycle progression from G1 to S phase while thidiazuron caused the halt there. Quantitative-PCR (qPCR) analysis revealed that tZ upregulated the expression of one cyclin like as higher plant system, whereas thidiazuron downregulated its expression. These results suggest that this cyclin plays a crucial role in controlling the cell cycle progression in *T. gondii* and concomitantly its proliferation. Since cytokinins are also known to regulate chloroplast development in plants, we examined whether cytokinins affect apicoplast, which is a homologous organella to chloroplast in *T. gondii*. Immunofluorescence microscopy and qPCR analysis revealed tZ increased the number of apicoplasts but thidiazuron extinguished apicoplast. These result suggest that cytokinins are very important molecules to regulate the cell cycle of the parasite and division of apicoplast.

Keynote Lecture II (Day 2: 8:45-9:45 on September 17)

Keynote lecture II

Evasive strategies of rabies virus

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Viruses are obligatory parasites. Successful completion of virus cycle and subsequent transmission to a new host relies upon the evolution of strategies that exploit the cellular machinery and modulate host cell signalling pathways, in particular, that governing premature cell death and promoting cell survival. Rabies virus, a neurotropic virus causing fatal encephalitis, is transmitted by saliva of an infected animal (mainly dogs but also bats) after bites or scratches. Rabies virus enters the nervous system through the neuromuscular junction via a motor neuron or through nerve spindles via a sensory nerve. Infecting neurons almost exclusively, it travels from one neuron to the next in the spinal cord to the brainstem, from where it reaches the salivary glands via cranial nerves. Once the rabies virus has reached the salivary glands, it is excreted in saliva and can be transmitted to a new host. After the successful completion of the virus cycle, death of the host occurs because of the exhaustion of the infected neurons accompanied with structural damages causing severe neuronal dysfunctions.

Intriguingly, once the rabies virus has entered the nervous system, its progression is not interrupted either by destruction of the infected neuron or by the immune response, the two major host mechanisms for combating viral infection. Rabies virus has two complementary characteristics particularly relevant to successful invasion of the nervous system: 1) the rabies virus escapes the host immune response and 2) protects the infected neurons against apoptosis or premature destruction of neurites.

Oral presentation I (Day 1: 10:45-11:05 on September 16)

O-1

The role of cholesterol in *C. burnetii* pathogenesis

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The gram-negative bacterium *Coxiella burnetii* is the causative agent of the zoonotic disease Q fever. A large human outbreak (>4,000 cases) associated with infected dairy goats is currently ongoing in the Netherlands, causing significant morbidity and economic loss. A better understanding of C. burnetii-host interactions is critical for the development of new Q fever control methods. Central to *C. burnetii* pathogenesis is establishment of its intracellular niche, a modified acidic phagolysosome, or parasitophorous vacuole (PV). The PV membrane is structurally rigid, impermeable to molecules <500 Da, fusogenic, and cholesterol-rich. To further understand the importance of cholesterol in the Coxiella PV, we utilized cholesterol-free DHCR24-/- mouse embryonic fibroblasts, which lack the Δ24 sterol reductase required for the final enzymatic step in cholesterol biosynthesis and consequently accumulate desmosterol in cellular membranes. Using these cells, we have shown cholesterol is not essential for Coxiella infection, PV formation, and bacterial growth. In addition, while possessing an active DHCR24-/- homolog, *C. burnetii* does not generate cholesterol de novo from host cell sterols. While these data suggest cholesterol is not essential for *C. burnetii* pathogenesis, cholesterol does appear to play a role in trafficking to the PV. We have found that ORP1L, a mammalian cholesterol-binding protein involved in endosomal trafficking, localizes to the PV. ORP1L on the *C. burnetii* PV co-localizes with the endoplasmic reticulum (ER), a novel finding suggesting the PV forms contact sites with the ER. Ongoing experiments are addressing the role of ORP1L in trafficking to the *C. burnetii* PV, as well as the significance of interactions between the PV and the ER.

Oral presentation I (Day 1: 11:05-11:25 on September 16)

O-2

Microbial manipulation of host transcriptional programming by nuclear toxins – the *Anaplasma phagocytophilum* AnkA model

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Anaplasma phagocytophilum, the causative agent of human granulocytic anaplasmosis (HGA), is an obligate intracellular bacterium of neutrophils. Upon infection, *A. phagocytophilum* alters neutrophil functions in part by altering transcription. Antimicrobial defense genes are down-regulated with infection, including *CYBB*, a critical component of the NADPH oxidase. AnkA, an *A. phagocytophilum* type 4 secretion system substrate, translocates to the nucleus of infected neutrophils, binds to AT-rich DNA in the +12 to -48 and -109 to -138 regions of the *CYBB* promoter and decreases *CYBB* transcription. When these AT-rich regions are mutated, AnkA no longer binds and *CYBB* silencing is reversed. Among host defense genes similarly affected are those physically clustered on chromosomes, suggesting that *A. phagocytophilum* induces chromatin remodeling. Chromatin immunoprecipitation shows hypoacetylation and hypermethylation of H3 around the promoters of *CYBB* and defense genes, consistent with their decreased transcription. Infection with *A. phagocytophilum* also associates with increased histone deactylase (HDAC) transcription, protein expression, and activity. HDAC inhibition by trichostatin A or sodium butyrate impairs intracellular bacterial replication, but this in unaffected by preincubation of the bacteria with these inhibitors. We hypothesize that AnkA either has i) endogenous HDAC activity, ii) antagonizes transcriptional regulator binding, or iii) recruits histone modifying complexes resulting in decreased transcription of defense genes. Using recombinant AnkA we determined that AnkA lacks HDAC activity. Moreover, AnkA co-immunoprecipitates with at least 2 nuclear proteins and has properties that mimic SATB1, a matrix attachment region (MAR)-binding protein. MARs are AT-rich regions of DNA critical for chromatin structure and transcription, and are predicted to be present where AnkA binds. This suggests the hypothesis that AnkA alters neutrophil functional programs via recruitment of histone modifying complexes in a manner similar to eukaryotic MAR-binding proteins. Work is underway to identify AnkA binding partners and binding sites across the genome to further elucidate mechanisms by which *A. phagocytophilum* controls host cell transcriptional programs.

Oral presentation I (Day 1: 11:25-11:40 on September 16)

O-3

Impact of the E540V Amino Acid Substitution in GyrB of *Mycobacterium tuberculosis* on Quinolone Resistance

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Introduction; Recently, the emergence of drug-resistant tuberculosis, multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) are becoming an increasing public health problem and poses a serious threat to world's TB control. The outbreak of MDR-TB leads fluoroquinolones (FQs) to becoming an important second-line anti-tuberculosis agent. The major target of FQs in *M. tuberculosis* is DNA gyrase consisted of two subunits GyrA and GyrB which form the catalytically active $GyrA_2GyrB_2$ heterotetrameric structure. The quinolone-gyrase interaction site is thought to be located at the so-called Quinolone Resistance-Determining Regions (QRDRs) in GyrA and GyrB where the majority of mutations conferring resistance to quinolones exist. Although the amino acid substitutions conferring quinolone resistance were mainly found within the QRDR in GyrA, some quinolone resistant *M. tuberculosis* strains carried mutations in GyrB. Recently, we found a mutation located at codon 540 of gyrB which caused amino acid substitution from Glutamic acid to Valine (E540V) from a clinical isolate in Bangladesh. Since little information is available on the relationship between the E540V mutation in GyrB and quinolone resistance, we expressed a mutant E540V, and compared their characteristics by in vitro enzyme assays.

Methods; Wild type (WT) and recombinant gyrases carrying E540V mutation in GyrB were expressed by an *E. coli* expression system. Enzymatic activities were assessed by ATP-dependent DNA supercoiling assay and the resistances of gyrases to quinolones were investigated with eight quinoloes (Gatifloxacin [GAX], Levofloxacin [LVX], Ciprofloxacin [CIP], Moxifloxacin [MXF], Norfloxacin [NOR], Enoxacin [ENX], Sparfloxacin [SPX], and Sitafloxacin [SIX]) by quinolone-inhibited supercoiling assay. The degree of resistance was calculated as IC50s (50% inhibitory concentrations).

Results; The mutant gyrase showed higher IC_{50}s than WT against eight quinolones, except in SIX. IC_{50}s against NOR and ENX were higher than 84 μg/ml in all test including WT. IC_{50}s of GAT, SPX and MXF, especially CIP (36-fold), were higher against E540V. IC_{50}s of SIX was higher against E540V than those for the WT, although the value was as low as 10 μg/ml.

Discussion; Obvious relationship between quinolone structure and the degree of resistance (increased IC_{50}s) of mutant gyrase possessing mutation in GyrB was observed. The GyrB-E540V mutant enzyme showed higher resistance against quinolones. We also demonstrated an association between structural features of quinolones and activities against the WT and E540V forms of the DNA GyrB subunit in *M. tuberculosis.* The interaction between FQs and GyrB, which is composed of hydrogen bonding networks involving substituent of FQs and amino acid residues of QBP in GyrB, plays an important role in the inhibitory activity of FQs.

Oral presentation I (Day 1: 11:40-11:55 on September 16)

O-4

Seroprevalence of anti-leptospira antibodies among patients with acute febrile illness with renal dysfunction in spite of negative result with several laboratorial leptospira tests in Thailand

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Leptospirosis is zoonotic disease of global importance. According to the report of WHO, more than 500,000 cases of severe leptospirosis are reported each year, with case fatality rates exceeding 10%. In addition to that, clinical fetures of leptospirosis are quite various from asymptomatic or mild flu-like symptom to fatal outcome, so actual disease fetures and burden have not been understood yet.

In the current situation, the diagnostic methods of leptospirosis are quite problematic with low sensitivity (culture, PCR), and late elevation of titer (microaggulutination test (MAT)), or low specifisity and sensitivity (another serological test).

Thiland is one of the most leptospirosis endemic nation. To control leptospirosis in endemic countries, we have to determine effective diagnostic methods and understand real epidemiological fetures.

Purpose: 1. To assess seroprevalence of anti-leptospira antibodies in epidemic area of leptospirosis, Thailand.
2. To know relationships among antibodies reactions to different antigens in patients with leptospirosis in Thailand.

Subjectives: 284 human sera were obtained from patients with acute febrile illness (AFI) and renal dysfunction whose serological tests of leptospirosis were determined negative in Surin province, Thailand in 2003 ~ 2004 . The serological test were performed in each hospital in Surine province and including leptospira latex agglutination test, indirect immunofluorescence assay (IFA), MAT, lateral flow.

Methods: We made 2 recombinant antigens which are related to pathogenesis of leptospirosis. The one is partially expressed LipL32, and the other is LigA. We confirmed that theses antigens have quite few polymorphisms among different serotypes and strains which are known as virulent pathogen against humans by website of GenBank®.

Patients' sera were tested for IgG and IgM antibodies against these antigens by enzyme linked immunosorbent assay (ELISA).

Results: Positive rate of LipL32-IgG is 63.7% and IgM is 51.8%.On the other hand positive rate of LigA-IgG is 48.6%, and IgM is 62.0%. By χ^2 analysis, LipL32-IgG,and IgM have strong correlation with LigA-IgM ($p<0.01$), and reverse correlation with LigA-IgG ($p=0.05, <.01$).

Conclusion: These results indicate that we have to carefully diagnose patients with AFI and renal dysfunction in epidemic area for leptospirosis even with result leptospira-MAT negative. The reciprocal variation of LigA-IgG might be caused by accumulation of former infection with leptospira, so it is expected to do further assessment of correlation between changes of these antibodies titer and clinical courses.

Oral presentation II (Day 1: 15:15-15:35 on September 16)

O-5

Home renovating: how malaria parasites export remodelling proteins into parasitized human erythrocytes

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Malaria parasites kill over 3000 people every day, predominantly children under five years old, and drug resistance is rapidly developing across endemic regions. The key to successful infection of vertebrate hosts is large-scale proliferation of parasites in the bloodstream. The survival of *Plasmodium falciparum* inside erythrocytes requires extensive renovation of the host cell by hundreds of exported parasite proteins. This remodeling is essential for the transfer of nutrients and waste, immune evasion by antigenic variation and disease pathology. Remodeling occurs *via* a protein trafficking network of parasite origin elaborated in the host cell. Malaria parasites employ a highly conserved N-terminal pentameric motif (RxLxE/Q/D) termed the *Plasmodium* export element (PEXEL) to target these hundreds of proteins beyond the encasing vacuole membrane into the host cell. We have used a variety of genetics and proteomics approaches to understand how the PEXEL facilitates protein trafficking. We show that the PEXEL is a proteolytic cleavage site that is cleaved by an endoplasmic reticulum-resident parasite protease. Furthermore, we demonstrate that cleavage of cargo proproteins specifically by this enzyme is critical for their correct sorting to the host cell. The identification of the parasite enzyme responsible for controlling export of over 300 virulence and survival proteins provides an exciting target for the design of new and much needed antimalarial drugs.

Oral presentation II (Day 1: 15:35-15:50 on September 16)

O-6

Regulatory dendritic cells expand and dampen pathogenicity during *Trypanosoma evansi* infection

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Trypanosoma evansi, the etiological agent of the livestock disease "Surra", causes significant economic losses to livestock industry around the world. *T. evansi* infection triggers inflammation in the hosts through the activation of immune cells leading to the elimination of the parasite. However, uncontrolled proinflammatory responses also contribute to the development of trypanosome infection-associated tissue injury. Therefore, we aimed at finding the balance between pro- and anti-inflammatory responses during the parasite infection to avoid tissue damage. To determine whether inflammatory cytokines or their receptors are involved in the pathogenicity in the late stage of *T. evansi* infection, PCR array analysis in the spleens of infected mice was performed. The expressions of the CCL8 and interleukin -10 (IL-10) transcripts were significantly increased in infected mice. These results suggest that the recently characterized subset of regulatory dendritic cells (DCs) that expresses low levels of CD11c and high levels of CD45RB would be expanded in infected mice. We therefore investigated the kinetics of regulatory DCs in *T. evansi*-infected mice. During the *T. evansi* infection, these DCs became prevalent and the expression of indoleamine 2,3 dioxygenase (IDO), which is one of the marker of regulatory DCs, was also up-regulated. Interestingly, the prolonged survival of *T. evansi*-infected mice was observed when these DCs were implanted into these mice. In this study, we showed that a subset of regulatory DCs are expanded and act as potential regulators of the inflammatory responses in infected mice. These DCs might have a preventive and therapeutic potential for the treatment of *T. evansi*-induced inflammation.

Oral presentation II (Day 1: 15:50-16:05 on September 16)

O-7

CD4$^+$Foxp3$^+$Regulatory T cells in hepatic *Leishmania donovani* persistence

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Visceral leishmaniasis (VL) is a chronic and fatal disease in humans and dogs caused by the intracellular protozoan parasites *Leishmania donovani* or *Leishmania infantum* (*chagasi*). The liver, spleen, bone marrow and lymph nodes are major sites of parasite growth, parasite persistence and disease pathology. In murine models of VL, organ-specific immune responses impact on the outcome of complex symptoms but host immune responses in the chronic phase and a role of lymph node in experimental VL are poorly understood. In the present study, we therefore employed alymphoplastic NF-κB inducing kinase mutant *aly/aly* mice, possessing lacking lymph nodes and Peyer's patches, as a model of study. The host immune responses, with special reference to the liver, were examined for up to 7 months after intravenous inoculation with *L. donovani* promastigotes; focusing on parasite burden, granuloma formation, expression of cytokines/ chemokines mRNA and induction of regulatory T cells (Treg). While control *aly/+* mice showed an early peak of hepatic parasite growth at 4 weeks post infection (WPI) and resolved the infection by 8 WPI, *aly/aly* mice showed a similar early peak in hepatic parasite burden but maintained a low -level chronic infection, which is associated with delayed and impaired granuloma maturation. Although hepatic CD4$^+$Foxp3$^+$ but not CD8$^+$Foxp3$^+$ T cells were first detected at 4 WPI in both strains of mice, the number of CD4$^+$Foxp3$^+$ T cells and accumulation of *Foxp3* mRNA were significantly increased in *aly/aly* mice from 8 WPI. Immunohistochemical analysis demonstrated the presence of Foxp3$^+$ T cells inside *L. donovani*-induced hepstic granulomas. Furthermore, treatment of infected-*aly/aly* mice with anti-CD25 or anti-FR4 mAb resulted in significant reductions in both hepatic Foxp3$^+$ cells and parasite burden. Thus, NIK mutation may modulate the liver-specific immunity to *L. donovani* infection, but does not affect the proliferation and function of Tregs. We provide the first evidence that CD4$^+$Foxp3$^+$ Tregs can mediate *L. donovani* persistence in the liver during murine VL, a result which will help to establish new strategies of immunotheraphy against this intracellular protozoan pathogen. Finally, this study could support a role of the liver as a back-up organ for priming cell-mediated immunity even if the secondary lymphoid organs are not functional.

Oral presentation II (Day 1: 16:05-16:20 on September 13)

O-8

Biological characterization of molecules from poultry red mite, *Dermanyssus gallinae* for the development of a new control method of the mite

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The red mite, *Dermanyssus gallinae*, is distributed worldwide, and is 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*).

First, we analyzed the vectorial role of the red mites for the transmission of various pathogens. Some bacteria and viruses, including zoonotic pathogens such as *Erysipelothrix* and *Pasteurella*, were detected from some red mite pools from a poultry farm by using PCR. These results show a possible vectorial role of red mite for the transmission of various pathogens, and the control of red mites were important for the prevention of zoonosis. However, little is known on the properties of the molecules of red mite, and acaricide-resistant mite is also widely present in farms, which could be a serious problem to poultry industry in the future. Therefore, in this study, a global analysis of gene expressions in red mite has been performed.

A plasmid cDNA library was constructed from the red mite collected from a poultry farm. A total of 1,066 expression sequence tags (ESTs) were sequenced from the library. When these sequences were compared to NCBI databases, 287 sequences were identified as the genes of known function. These clones include ones which have similarities with acaricide target genes, drug-metabolizing enzymes, and vaccine candidates.

Among them, we focused on the peroxiredoxin (Prx4) cDNA clone, which is suggested as a possible vaccine candidate for other ticks, and function analysis of this molecule was performed. Currently, functional analyses of other cDNA clones are in progress to identify new vaccine candidates and to identify target molecules involved in the acaricide-resistant mechanism.

Oral presentation III (Day 2: 9:55-10:15 on September 17)

O-9

E2 Antibody inhibits nonstructural protein processing and blocks intracellular Alphavirus replication

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Alphaviruses are important causes of encephalomyelitis in humans and equids. Due to the nonrenewable nature of neurons, the immune response must control virus multiplication in mature neurons by a nonlytic mechanism during recovery. Both innate and adaptive immune responses orchestrate virus clearance. Sindbis virus provides a tractable model for this disease. Monoclonal antibody to the Sindbis virus envelope protein E2 (α-E2 MAb) directs viral clearance in both SCID mice and primary neuronal cultures. To further define the mechanism by which antibody directs cellular clearance of Sindbis virus, we are taking advantage of 2 cultured rat neuronal cell lines, AP-7 and CSM14.1 cells, that differentiate upon temperature shift. Treatment of differentiated neurons with α-E2 MAb, which is retained on the cell surface, reduced virus multiplication in a dose dependent manner and improved neuronal survival after infection. There was no effect upon treatment with antibody to the other viral envelope protein, E1.

Alphaviruses encode two polyproteins that are cleaved to form the nonstructural and structural viral proteins. Cleavage of the non-structural polyprotein occurs in distinct steps to regulate genome replication and structural protein production. α-E2 MAb treatment blocked processing of the non-structural polyprotein, but not the structural polyprotein. We hypothesize that crosslinking of α-E2 MAb to E2 on the surface of infected neurons results in intracellular signaling that blocks nonstructural polyprotein processing and subsequently limits Sindbis virus multiplication. These studies will lead to a greater understanding of how mature neurons control virus multiplication and identify antiviral targets for development of antivirals.

Oral presentation III (Day 2: 10:15-10:35 on September 17)

O-10

Non-neutralizing antibodies protect against lethal West Nile virus infection via Fc-gamma receptor and complement–dependent mechanisms

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West Nile virus (WNV) infection can progress to severe neuroinvasive disease in humans, especially in the elderly and immunocompromised. Studies in mice lacking B cells have demonstrated the essential role of antibody in protection against WNV infection. Indeed, passive transfer of monoclonal (MAbs) or polyclonal antibodies before or after infection protects animals from WNV disease. The human antibody response to flavivirus infection is dominantly directed toward a cross-reactive epitope, the fusion peptide in domain II (DII) of the envelope protein. We have shown that passive transfer of MAbs against this epitope is protective in mice, even though they recognize only a subset of virions and do not neutralize infection significantly in vitro. We establish that antibodies with poor intrinsic neutralizing capacity against several epitopes strongly protect wild type mice. However, these MAbs fail to protect congenic mice deficient in both activating Fc-gamma receptors (FcγR) and the complement opsonin C1q. Further, antibodies lacking Fc effector functions no longer protect wild type mice. FcγRI (CD64) and FcγRIII (CD16) are key effector molecules that associate with survival benefit based on antibody isotype. Non-neutralizing antibodies do not require T, B, or NK cells to protect from WNV. Rather, pre-treatment with non-neutralizing antibodies decreases viral load in the serum and brain, an effect which is dependent upon phagocytic cells. We are currently investigating which populations of phagocytes are responsible for the protective effects of non-neutralizing MAbs. Overall, these studies contribute to our understanding the functional significance of the polyclonal antibody response of flavivirus-infected humans, which is largely directed toward epitopes that are recognized by non-neutralizing antibodies. This work also highlights the limitations of current in vitro neutralization assays that are used routinely in vaccine development as surrogate markers of protection, as they do not account for beneficial effects of immunodominant non-neutralizing antibodies.

Oral presentation III (Day 2: 10:35-10:50 on September 17)

O-11

The K627E amino acid substitution of the PB2 of A/Hong Kong/483/1997 (H5N1) influenza virus alters the efficiency of RNA synthesis of NP gene

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Influenza virus is enveloped and has eight negative-sense viral RNA (vRNA) gene segments which are packaged into viral ribonucleoprotein complexes. The viral RNA polymerase is a heterotrimeric complex of PB2, PB1, and PA proteins. This complex synthesizes virus mRNA, cRNA, and vRNA. The role of each protein involved in the regulation of RNA synthesis is yet unclear.

In the present study, the effect of amino acid substitution of Lysine for Glutamic acid at position 627 (K627E) of the PB2 of A/Hong Kong/483/1997 (H5N1) (HK483-K) in the RNA synthesis activity was examined. The mutant virus HK483-E was generated by using site-directed mutagenesis and reverse genetics from HK483-K. The amino acid substitution of the PB2 did not affect the virus growth in 293T cells. To examine the affect on the RNA synthesis activity of the virus, the amounts of vRNA, cRNA, and mRNA of each gene in 293T cells infected with HK483-K or HK483-E were measured by primer extension assay. The relative amounts of mRNA to vRNA of the PB2, PB1, PA, HA, and the NA of HK483-E were lower compared with those of HK483-K. On the other hand, the relative amount of mRNA to vRNA of the NP of HK483-E was higher compared with that of HK483-K. The present results suggest that the K627E of the PB2 of HK483-K affected the RNA synthesis starting from the vRNA promoter of the NP gene of those viruses. Since the amino acid substitution of HK483-K was responsible for the pathogenicity against mouse, the comparison of the RNA synthesis efficiency in the mouse cells is under way.

Oral presentation III (Day 2: 10:50-11:05 on September 17)

O-12

A single amino acid residue in major histocompatibility complex class I is a determinant for its function as an equine herpesvirus-1 receptor

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Equine herpesvirus-1 (EHV-1), an alphaherpesvirus of family Herpesviridae, is the causative agent of respiratory disease, abortion, and a serious neurologic disease known as encephalomyelitis in horses. EHV-1 utilizes equine major histocompatibility complex (MHC) class I molecules as entry receptors. However, hamster MHC class I molecules on EHV-1-susceptible CHO-K1 cells play no role in EHV-1 entry. To identify the region of the MHC class I molecules responsible for EHV-1 entry, domain exchange and site-directed mutagenesis experiments were performed, in which parts of the extracellular region in the hamster MHC class I molecule (clone C5) were replaced with corresponding sequences from the equine MHC class I molecule (clone A68). Substitution of alanine for glutamine at position 173 (Q173A) within the α2 domain of the MHC class I molecule enabled hamster MHC class I C5 to mediate EHV-1 entry into cells. Conversely, substitution of glutamine for alanine at position 173 (A173Q) in equine MHC class I A68 resulted in loss of the EHV-1 receptor function. Further mutational analysis showed that hydrophobicity of the amino acid at position 173 of MHC class I is involved in EHV-1 receptor function. In addition, equine MHC class I clone 3.4, which possess hydrophilic threonine at position 173, was unable to act as an EHV-1 receptor. Substitution of hydrophobic alanine for threonine at position 173 (T173A) also enabled MHC class I 3.4 to mediate EHV-1 entry into cells. These results revealed that a subset of equine MHC class I molecules act as EHV-1 receptors and suggested that hydrophobicity of the amino acid at position 173 of MHC class I is one of the determinants for its EHV-1 receptor function.

Oral presentation III (Day 2: 11:05-11:20 on September 17)

O-13

Detection of newly generated PrP^{Sc} in Neuro2a cells inoculated with fluorescent-dye labeled purified PrP^{Sc}

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Background

Molecular mechanism of prion propagation has been analyzed with cells persistently infected with prion, however, events required for establishment of prion infection, especially on the early phase after prion inoculation, are poorly understood.

Objectives

The purpose of this study is to identify subcellular compartments where de novo synthesis of PrP^{Sc} takes place and to characterize the route of trafficking of exogenously introduced PrP^{Sc} to the compartments after inoculation of PrP^{Sc}, major component of prions.

Methods

PrP^{Sc} purified from brains of mice infected with 22L prion strain was labeled with Alexa Fluor-555 succinimidyl ester. The labeled PrP^{Sc} was sonicated and inoculated into a Neuro2a subclone, N2a-3. Trafficking of the labeled PrP^{Sc} was analyzed by time-lapse imaging. Newly generated PrP^{Sc} was analyzed by indirect immunofluorescence assay using mAb 132 and Alexa Fluor 488-labeled secondary antibodies. For the detection of PrP^{Sc} with mAb 132, fixed cells were treated with guanidinium thiocyanate prior to immunostaining.

Results and Discussion

Using Alexa Fluor 555-labeled PrP^{Sc} and mAb 132, the inoculated PrP^{Sc} could be distinguished from newly generated PrP^{Sc} with following criteria: the inoculated PrP^{Sc} was detected both with Alexa Fluor 555 (directly coupled to purified PrP^{Sc}) and mAb 132 (indirect immunostaining), while newly generated PrP^{Sc} was detected only with mAb 132. Time-lapse imaging revealed that inoculated PrP^{Sc} was dynamically transported throughout the cells 1day after inoculation. Three days after inoculation, newly generated PrP^{Sc} as well as the inoculated PrP^{Sc} could be simultaneously detected at peri-nuclear region of the same cell. Thereafter, newly synthesized PrP^{Sc} was continuously detected at peri-nuclear region similarly to persistently infected cells. Although inoculated PrP^{Sc} moved around within the cells including neuritic-like structures, some particles appeared to be transported to peri-nuclear regions. These results suggest that the pathway to peri-nuclear region is closely associated with the establishment of productive prion infection.

Oral presentation IV (Day 2: 13:00-13:20 on September 17)

O-14

Hantavirus infection in the Southeast of Brazil

Glauciane Garcia de Figueiredo, Alex Martins Machado, Gilberto dos Santos Sabino Júnior, Gelse Mazzoni Campos, Alessandra Abel Borges, Marcos Lázaro Moreli, Fabiano Pinto Saggioro, Luiz Tadeu Moraes Figueiredo
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Hantavirus are enveloped negative-sense RNA *Bunyaviridae.* These are zoonotic rodent-borne viruses that occur in the whole world. Hantaviruses in the American continent are causative agents of a severe human disease, the Hantavirus cardiopulmonary syndrome (HCPS). In the Southeast of Brazil, the newly isolated Araraquara virus (ARAV) is an important causative of HCPS. This part of the country is highly populated and has suffered and intensive degradation of the forest and cerrado areas (savannah) for cattle and agriculture farming that includes an extensive sugar cane monoculture. This degraded environment has enhanced human contact with wild rodents and transmission of hantaviruses. Besides, most of the population do not know about HCPS and are not attempt on how to avoid rodent contact and hantavirus infection. It is known that the rodent-host for ARAV is the *Muridae, Sygmodontinae, Necromys lasiurus*. Hantavirus is transmitted to humans via aerosolized excreta of chronically infected carrier rodents. The aim of this work is to show aspects of hantavirus epidemiology and infection in the region. Between 1998 thru 2011, we have analyzed 184 HCPS suspect cases and were able to confirm this diagnosis in 65 (35.3%). The majority were male gender (81.5%) and rural workers (43%). However, regarding to HCPS cases occurring in peri-urban areas the population of risk are women and children. This severe disease produced a 44.6% case fatality. HCPS is seasonal in the region, occurring mostly during the dry season (April to August). Sanitary agency authorities and the medical community are becoming more aware to hantaviral epidemiology and more aware to HCPS suspected cases. It, probably, is beginning to reduce the case fatality of HCPS in the region in the last years.

Oral presentation IV
(Day 2: 13:20-13:35 on September 17)

O-15

Investigation and phylogenetic analysis of novel arenaviruses in Zambia

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In Africa, Old World Arenavirus (OWA) that have not been reported to be pathogenic to humans have been found in Central Africa (Ippy and Mobala viruses), Eastern Africa (Morogoro and Mopeia viruses), and South Africa (Merino Walk virus). Before 2008, only 1 OWA, the Lassa virus, which is exclusively found in West Africa, was known to cause hemorrhagic fever. In September 2008, a patient in Zambia developed hemorrhagic fever and, after transport to South Africa, 4 people are transmitted the infection nosocomially. Four of these 5 infected patients died. The hemorrhagic fever was caused by a novel arenavirus named Lujo virus. The Lujo virus belongs to the OWA group based on geography but is genetically slightly divergent from other OWAs.

Natural reservoirs of OWAs are rodents of the genus Murinae. To reveal epizootiological aspects of arenavirus in Zambia, a molecular surveillance of arenavirus in rodents was carried out in the Republic of Zambia in 2009. In total of 263 rodents were collected in Mfuwe, Namwala and Lusaka. In total, 263 rodents were collected from Mfuwe, Namwala, and Lusaka. Five of the 263 RNA samples extracted from kidneys were positive for arenavirus, as determined by the one-step RT-PCR. Two representative viruses from Namwala and Lusaka were determined the full genome sequence. Phylogenetic analysis and calculated genetic distances among Old World Arenaviruses indicated that the Zambian strains are related to the Mobala, Morogoro, and Mopeia viruses. Sequencing of the rodent gene encoding cytochrome b revealed that the reservoir host was Mastomys natalensis, which is also the reservoir of Lassa and Mopeia viruses. The novel Zambian strain described here has been tentatively named Luna (Lusaka-Namwala) virus. The Luna virus was isolated from a kidney in a rodent captured in Lusaka. Transmission electron microscopy showed that the isolated Luna virus possesses typical morphological characteristics.

Oral presentation IV
(Day 2: 13:35-13:55 on September 17)

O-16

Modelling the influence of habitat hot spots on the disease spread and vaccination strategies

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"The spread of disease in wildlife populations depends strongly on rates of contact between hosts. Resource "hotspots", places regularly visited by large numbers of individuals or social groups, can substantially alter contact rates and, hence, disease propagation. However, few studies have addressed this question in wild animal populations. We developed a spatially-explicit simulation model motivated by the transmission of Ebola virus in networks of gorilla social groups that visit mineral rich forest clearings. In this lattice model, social groups visit a hotspot at a rate inversely proportional to its distance from their home-range centroid. We first explored disease dynamics in a simple Susceptible-Infectious -Removed (SIR) model, where individuals could be infected either by other individuals within their group, by neighbouring groups, and when crossing other groups' home-ranges on their way to the hot spot. Our model predicts that hot spots may dramatically increase the percolation probability of a disease, especially in poorly connected populations. Moreover, it reveals that hot spots can strongly affect even groups who never visit them. Counter-intuitively, groups ranging far from the hot spot can become infected earlier and have a higher attack rate than those ranging close to the hot spot. Finally, we compared the efficiency of vaccination strategies in which either randomly chosen groups were vaccinated or groups at or near the hotspot were preferentially targeted. We relate these modelling results to ongoing Ebola vaccine trials in captive and wild apes."

Oral presentation IV (Day 2: 13:55-14:10 on September 17)

O-17

Filovirus-specific antibodies detected in fruit bats and nonhuman primates in Zambia

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Ebola virus (EBOV) and Marburg virus (MARV) belong to the family *Filoviridae* and cause severe hemorrhagic fever in primates. Despite the recent discovery of Reston EBOV in domestic pigs in the Philippines and of fruit bat species as potential reservoires for EBOV and MARV, the search for the absolute reservoirs and potential amplifying hosts remains ongoing. Although sporadic outbreaks of Ebola and Marburg hemorrhagic fever have been reported in central African countries, there has been no information on the epidemiology in Zambia surrounded by the countries where outbreaks of Ebola or Marburg hemorrhagic fever have occured. We collected serum and tissue samples from 432 fruit bats (*Eidolon helvum*) between 2006 and 2010 and 243 nonhuman primates (*Chlorocebus pygerythrus* and *Papio ursinus*) between 2008 and 2010 in Zambia, and filovirus-specific antibody prevalence and presence of filovirus RNA genome were investigated. We found that 79 (18.3%) bats had filovirus-specific IgG antibodies. IgG antibodies specific to Zaire EBOV, Sudan EBOV, Côte d'Ivoire EBOV, Bundibugyo EBOV, Reston EBOV, and MARV were detected in 4.4%, 2.8%, 3.7% 2.5%, 2.3%, and 2.5% of the bats examined, respectively ($P < 0.01$). So far filovirus RNA was not detected in their tissue and serum samples. In nonhuman primates, the prevalence of the specific IgG antibody was significantly higher than expected (32.9%). IgG antibodies specific to Zaire EBOV, Sudan EBOV, Cote d'Ivoire EBOV, Bundibugyo EBOV, Reston EBOV, and MARV were detected in 8.6%, 6.6%, 3.7%, 7.0%, 4.5%, and 2.5% of the primates examined, respectively. Filovirus-specific IgM antibodies were also found in the sera of some primates, but no filovirus RNA was detected in their spleen samples. These findings provide new insights into filovirus epidemiology in Zambia and suggest the potential risk for a human outbreak in Zambia.

Oral presentation V (Day 2: 14:30-14:50 on September 17)

O-18

H5N1 virus in Russia (2005-2011): molecular epidemiology, ecology and evolution.

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The report focuses on some epidemiological, ecological and evolutionary aspects of H5N1 virus in Russia from the first detection till now.

The first influenza A (H5N1) outbreak in Russia was reported in the summer of 2005 in the territory of Western Siberia (Lipatov etal., 2007). With migratory wild birds, virus spread westward across Eurasia and as far west as England and West Africa. Analysis of the nucleotide sequence of the hemagglutinin (HA) gene showed that strains isolated in Russia in 2005 were related phylogenetically to H5N1 viruses that had caused an outbreak among wild birds at Qinghai Lake in China in Spring 2005. Subsequently, the Qinghai-like (clade 2.2) HPAI H5N1 lineage was detected in wild birds and poultry in many countries. The first case of Fujian sub-clade 2.3.2 influenza virus (H5N1) lineage in the Russian Far East was recorded in April 2008. In June 2009, an outbreak of HPAI was recorded in wild birds in Mongolia and on the Uvs-Nuur Lake in Russia. Phylogenetic analysis of HA gene showed that viruses belong to clade 2.3.2. These viruses were close to viruses isolated in Mongolia at the same time and in Japan (Hokkaido) in 2008. We believe that the virus isolated in 2009 from Uvs-Nuur Lake was probably introduced by wild birds that wintered in Southeast Asia.

We hypothesized that bodies of water like the Qinghai Lake and the Uvs Nuur Lake may play an important role in the circulation of avian influenza so we suggested enhancing of surveillance program in this area and therefore we continued to study new outbreaks thoroughly (Sharshov et al., 2010).

Our hypothesis was confirmed in June 2010. An outbreak of HPAI was recorded in wild birds at the Uvs Nuur Lake (OIE, 2010). Phylogenetic analysis of the hemagglutinin (HA) gene showed a close relation to strains isolated during outbreaks at the same location in 2009, at the Qinghai Lake in 2009 and in Mongolia in 2010 as all of them fall into clade 2.3.2. We can suppose that these strains originally appeared before or around 2009 at the Qinghai Lake and later in May 2010 caused an outbreak in Central Mongolia (Sakoda et al., 2010). The report contains detailed comparative virological, molecular, pathogenic characteristic H5N1viruses isolated in Russia (2005-2011). Our experience with the 2005-2007 outbreaks and their HPAI H5N1 (clade 2.2) viruses originally appearing around Qinghai lake and then spreading first to Russia and later to Europe and Africa (Hars et al., 2008; Alexander and Brown, 2009) leads us to the assumption of a similar scenario for clade 2.3.2 HPAI virus global spread. Recent detection of clade 2.3.2 H5N1 viruses during outbreaks in Romania and Bulgaria (Reid et al., 2010) confirm this hypothesis. Furthermore 2.3.2 viruses have been detected in wild birds in Japan (2011 Feb; WHO). So, further investigation of the global distribution of clade 2.3.2 and 2.2 HPAI viruses will prove invaluable to give us a better understanding of the Evolutionary Ecology of avian influenza viruses in natural host populations.

This work was supported by Russian Federal Program (grants 16.740.11.0179 and 14.740.11.0247).

Oral presentation V (Day 2: 14:50-15:05 on September 17)

O-19

Reduction of Sampling Bias in Large Datasets by Systematic Trimming of Phylogenetic Trees

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The rapid development of molecular biology has accelerated the accumulation of genetic information on zoonotic pathogens. Phylogenetic analysis is one of the successful applications of these sequence data, and has been utilized to reveal the transmission route of pathogens. In the mean time, however, such massive sequence data yield enormously large phylogenetic trees, causing the difficulty in subsequent analyses. Moreover, since different numbers of sequences were submitted to the database from different countries, the dataset could not be considered as randomly selected samples. Thus there is an urgent need to solve these problems.

Here we propose a novel algorithm that trims branches in a large phylogenetic tree. Given a large phylogenetic tree, the algorithm outputs a compact form of the tree that has the similar topology of the original one. The fundamental idea of the algorithm is that a branch that has a similar sequence to another should reside near those on the phylogenetic tree. Given a phylogenetic tree with a large number of branches, at each step the algorithm finds the most similar pair of siblings and removes the branch that is longer than the other. Iterating this procedure until the number of trimmed leaves reaches the given threshold, one can obtain a compact form of the original phylogenetic tree.

We applied our algorithm to the phylogenetic analyses of hemagglutinins of influenza A viruses. Comparing strains in the trimmed phylogenetic tree with those in the original tree, we observed the change in the distribution of the locations where viruses were isolated. As the number of trimmed strains increases, a few locations account for much higher fractions. This change could be attributed to the sampling bias in the original dataset. By its nature, the original dataset contains a large number of sequences of viruses from the regions where surveillance are extensively conducted. This suggests that our algorithm could be useful to reduce such sampling bias in the original dataset.

Oral presentation V (Day 2: 15:05-15:25 on September 17)

O-20

Unlocking clues to influenza species specificity, virus transmissibility, and antiviral evasion through computational investigations of neuraminidase

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Understanding the structural dynamics of the influenza glycoproteins has been a long-standing goal due to its direct impact on public health. Such studies are especially important considering that the threat of species crossover of H5N1 avian influenza into the human population could lead to a devastating worldwide pandemic. We have used state-of-the-art computational biophysical simulations to characterize multiple components of the influenza glycoprotein neuraminidase (NA) that may be related to species specificity, virus transmissibility, and severity. In this talk I will address motion in the so-called 150-loop and the effects of this loop motion on cavity formation and resulting ligand / receptor binding capacity. Implications for antiviral drug design and host receptor recognition will be discussed. A second outstanding question that I will address is the role of an alternative binding site for the natural substrate, sialic acid, on the surface of NA. The conservation of residues surrounding this secondary site in avian strains, but not human or swine, suggested that this feature may play an unknown biological role in the avian subtypes. Importantly, the circulating pandemic H1N1 strain with proposed swine origin appears to have retained some of the key features of the secondary sialic acid site. The implications for influenza transmissibility and severity will be discussed.

Oral presentation V (Day 2: 15:25-15:45 on September 17)

O-21

Single particle reconstruction and assembly of Rift Valley fever virus

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Rift Valley fever virus (RVFV) is a member of the Bunyaviridae family (genus Phlebovirus) and is endemic through much of Africa with recent outbreaks in parts of the Arabian Peninsula. The virus is transmitted by mosquitoes and provokes disastrous outbreaks in a wide range of vertebrate hosts, man and cattle being the most sensitive ones. Infections in humans can lead to hepatitis or encephalitis and fatal hemorrhagic fever.

RVFV is an enveloped virus and has 4 major structural proteins – a nucleoprotein, two glycoproteins (Gn, Gc), and the RNA-dependent RNA polymerase. We determined the three-dimensional structure of RVFV by cryo-electron microscopy and could show that the two transmembrane viral glycoproteins were found to form cylindrical hollow spikes that cluster into distinct capsomers. The capsomers are arranged on the virus surface on an icosahedral lattice with a triangulation number of 12, representing a novel organization, so far only described for RVFV and Uukuniemi virus, another phlebovirus. However, little is still known about the structural organization and physical interactions of Gn and Gc within the virion and with the host-cell. The long term goals of our studies are to better understand the molecular mechanisms driving bunyavirus assembly from their component glycoproteins, the role Gn and Gc play during virus entry and to identify drug and vaccine targets to effectively combat highly virulent bunyaviruses. This information could potentially be exploited to develop vaccines and anti-viral therapeutics for this highly pathogenic virus.

Oral presentation V (Day 2: 15:45-16:00 on September 17)

O-22

Evaluation of the policies in the foot-and-mouth disease outbreak in Japan, 2010, and assessment of future outbreak risks

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(Background) The first outbreak of foot-and-mouth disease (FMD) occurred in April, 2010 in Miyazaki Prefecture. This was the first case in Japan for 10 years. It took approximately three months to control the whole outbreak using vaccination as well as "Stamping-out" and movement restriction policies. However, though the control activities were implemented, a total of 292 infected premises were reported and a total of 288,480 of cattle and pigs were destroyed, of which 76,872 animals were culled without infection suspicion. Total damages of this outbreak were much higher than for the last FMD outbreak in Japan in 2000. Several reasons why the control activities were not effective enough to control the outbreak quickly have been proposed. These hypotheses will be further investigated in this project.
(Objectives) The objectives of the present research are: 1) To map and assess control activities using a fault tree model. 2) To develop a simulation model able to replicate FMD outbreaks in Japan. 3) To explore the effectiveness of control measures limiting an FMD outbreak with minimum damage and to make recommendatins for the optimised management of future outbreaks
(Methods) A fault tree model is being developed which will map all activities and events where things can go wrong during an FMD outbreak. This will allow for the identification of critical event(s) that can limit the effectiveness of control activities and lead to disease spread. The fault tree will initially be generic. In a second step, it will be adapted to the Japanese situation of 2011. Comparing with the real data, we will assess the influence of specific events on the outcome and propose optimized control strategies.

In a second approach, we will use a transmission model to assess the effectiveness of control policies against FMD. We will use an existing disease simulation model developed in Univeristy of California, Davis, and parameterise it with Japanese data, where available. The output of the disease model is an estimation of the disease spread and its consequences. Alternative disease control scenarios will be formulated based on the results of the fault tree analysis. Their effect will be assessed in terms of duration of the outbreak, number of infected farms and number of culled animals. Recommendations will be made for future FMD control strategies.

P-1 **Characterization of antimicrobial resistance and class 1 integrons in *Escherichia coli* isolated from shrimp (*Litopenaeus vannamei*)**
Kanjana Changkaew

P-2 **Molecular characterization of multidrug-resistant *mycobacterium tuberculosis* isolates from Nepal**
Ajay Poudel

P-3 **Role of NF-kappa B signal inhibitor A20 and ABIN-3 in regression of TDM-induced tuberculoid granuloma in mice**
Yusuke Sakai

P-4 **An analysis of hospital-based sentinel surveillance data on leptospirosis in Sri Lanka, 2005 - 2008**
Chandika D. Gamage

P-5 **Genotying of *Ehrlichia ruminantium* by multiple-locus variable-number tandem-repeat analysis**
Ryo Nakao

P-6 **Whole genome next-generation sequencing reveals genome-wide nucleotide level polymorphisms in *Theileria parva***
Kyoko Hayashida

P-7 **Population genetic analysis and sub-structuring of *Theileria parva* in the northern and eastern parts of Zambia**
Walter Muleya

P-8 **Development of a molecular diagnostic method for differentiation and quantification of *T. orientalis* Genotypes**
Joseph Kamau

P-9 **Molecular detection and sequence characterization of three novel piroplasm spp from wild felids in Kenya**
Naftaly Githaka

P-10 **Molecular epidemiological studies on Animal Trypanosomiases in Ghana**
Jesca Nakayima

P-11 **Population genetic analysis of *Trypanosoma evansi* in the Sudan**
Bashir Salim

P-12 **Modeling Cutaneous Leishmaniasis Transmission in Costa Rica**
Luis Fernando Chaves

P-13 **Reaction of complement factors varies with prion strains in vitro and in vivo**
Rie Hasebe

P-14 **Prion-infected neurospheres as ex vivo models for investigation of piron-induced neurodegeneration**
Yukiko Sassa

P-15 **Surveillance of Marek's disease virus in migratory and sedentary birds in Hokkaido, Japan**
Shiro Murata

P-16 **The identification of the rodent reservoir of Montano virus, a novel hantavirus in Mexico**
Ngonda Saasa

P-17 **Development of immunochromatography strip-test for detecting of anti-hantavirus antibody in human and rat sera**
Takako Amada

P-18 **Cytotoxic T lymphocyte (CTL) responses to hantavirus in laboratory and wild rats**
Shumpei P. Yasuda

P-19 **Analysis of pulmonary edema in hantavirus-infected SCID mouse**
Takaaki Koma

P-20 **Development of the lethal animal model of human hantavirus infection**
Takahiro Seto

P-21 **Isolation of Hokkaido virus cultured cells derived from the kidney of gray red-backed vole (*Myodes rufocanus*)**
Takahiro Sanada

P-22 **Role of hantavirus nucleocapsid protein in intracellular traffic of glycoproteins**
Kenta Shimizu

P-23 **Construction and Characterization of Chimeric Virus between Tick-Borne Encephalitis Virus and Omsk Hemorrhagic Fever Virus**
Kentaro Yoshii

P-24 **Steric shielding of host proteins by filovirus glycoproteins**
Osamu Noyori

P-25 **Functional role of TIMP-1 after influenza A virus infection**
Yosuke Nakayama

P-26 **An H5N1 highly pathogenic avian influenza virus that invaded Japan through waterfowl migration**
Masahiro Kajihara

P-27 **Characterization of avian influenza viruses isolated from domestic ducks in Vietnam in 2009 and 2010**
Naoki Nomura

P-28 **Comparison of potency of whole virus particle and ether split pandemic influenza vaccine prepared from A/swine/Hokkaido/2/1981 (H1N1)**
Masatoshi Okamatsu

P-29 **Potency of an inactivated avian influenza vaccine prepared from a non-pathogenic H5N1 virus against the challenge with an antigenically drifted highly pathogenic avian influenza virus of clade 2.3.4**
Shintaro Shichinohe

P-30 **Rapid replication of H7 highly pathogenic avian influenza virus induces hyper expression of cytokine mRNA in chickens**
Saya Kuribayashi

P-31 **Cross-reactibity of HA-specific antibodies induced by immunization with inactivated influenza viruses in mice**
Mieko Muramatsu

P-32 **Cross-protective peptide vaccine against influenza A viruses developed in HLA-A*2402 human immunity model**
Toru Ichihashi

P-33 **Pathological examination of lung tissues in influenza A virus-infected aged mice**
Nilton Akio Muto

P-34 **Studies on the synthesis and expression of viral glycoproteins from Borna disease virus and avian bornavirus in transfected cell lines**
Yayoi Otsuka

P-35 **Detection of Polyomaviruses from Vervet monkey and Baboon in Zambia**
Hiroki Yamaguchi

P-36 **Possible origin of CSF antibodies induced by intrathecal immunization and apply to rabies control in experimental animals**
Yuji Sunden

P-37 **Chemokine improved anti-neurotropic virus immune response by attracting antibody secreting cells to the CNS**
Hyunkyoung Lee

P-38 **Characterization of protooncogene TCL1b as an Akt kinase co-activator**
Manabu Hashimoto

P-39 **Molecular characterization of immunoinhibitory factors PD-1/PD-L1**
Ryoyo Ikebuchi

P-40 **The role of non-receptor tyrosine kinase TYK2 in Toll-like receptor signaling**
Makoto Kuroda

Abstract

Poster Core time I at Lecture room 3 (Day 1: 16:30-17:30 on September 16)

P-1

Characterization of antimicrobial resistance and class 1 integrons in *Escherichia coli* isolated from shrimp (*Litopenaeus vannamei*)

Kanjana Changkaew[1], Fuangfa Utrarachkij[2], Kanokrat Siripanichgon[2], Chie Nakajima[1], Orasa Suthienkul[2] and Yasuhiko Suzuki[1]

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A total of 312 *Escherichia coli* were previously collected from samples in shrimp farms and markets in a province, located in southern Thailand. One hundred and forty-nine samples obtained from shrimp farms were fresh shrimp (27), surface water (54), shrimp waste (55), shrimp pond sediment (11), and shrimp feed (2). Additionally, 163 *E. coli* isolates obtained from the markets were fresh shrimps (37) and stilled water (126). All *E. coli* isolates were determined for susceptibility to 10 antimicrobial agents, extended-spectrum β-lactamase (ESBL), and class 1 integrons. Results showed that 17.6% (55/312) of all isolates were resistant at least one of 10 tested antimicrobial agents. Tetracycline resistant *E. coli* were found to be the highest prevalence (14.4%; 45/312). The *E. coli* resistant isolates were found to be highest in water (8.9%; 28/312) followed by shrimp waste (4.5%; 14/312), and fresh shrimp (4.2%; 13/312). Detection rate of resistant isolates from the markets (17.8%; 29/163) was not significantly higher than those from the shrimp farms (14.8%, 12/81) ($p > 0.05$). Multidrug-resistance (MDR) phenotype of *E. coli* (67.3%, 37/55) was significantly higher than those of single resistance pattern (32.7%, 18/55) ($p < 0.05$). Interestingly, only one ESBL producing strain was found in a shrimp waste sample and had quintuple resistant phenotype. Furthermore, 16 of 55 (29.1%) resistant isolates carried the integrase 1 gene and 7 of 16 (43.8%) isolates gave 3 different PCR amplicon profiles using primers from the variable region of integron. Extensive sequencing analysis of these 7 PCR amplicons revealed 4 types of gene cassettes including *dfrA17*and *aadA5* (4 isolates) *dfrA12* and *aadA2* (3 isolates). In addition, two insertion sequences elements (IS*26*, IS*5075*) were found in the variable region of class 1 integrons. In this study, the occurrence of MDR *E. coli* and class 1 integrons strains in shrimp and environmental samples indicated an urgent need for monitoring the emerging of antimicrobial resistant *E. coli* in shrimp farms and markets, in order to control the dissemination of antimicrobial resistant *E. coli* and further spread of resistant genes to other bacteria in the same environment.

Poster Core time II at Lecture room 3 (Day 2: 16:15-17:15 on September 17)

P-2

Molecular Characterization of Multidrug-Resistant *Mycobacterium tuberculosis* Isolates from Nepal

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Understanding of molecular basis of drug-resistance in *Mycobacterium tuberculosis* is important to develop rapid genotypic approaches for early diagnosis of multidrug- resistant (MDR) and extensively drug-resistant (XDR) tuberculosis. In the present study, we evaluated the nature and frequency of mutations associated to resistance in 52 rifampicin-resistant (RIF^r) and 55 isoniazide-resistant (INH^r) *M. tuberculosis* isolates collected from Nepal. Fourteen different mutations were identified in 49 of 52 (94.3%) RIF^r isolates. Single nucleotide substitution in the codons 531 (40.4%), 526 (25%) and 516 (19.2%) were the most prevalent mutations. Of 55 INH^r isolates, mutations in *kat*G and *inh*A promoter region were identified in 50 (90.9%) and 3 (3.6%) isolates, respectively. Forty-eight (87.3%) INH^r isolates exhibited nucleotide substitutions at codon 315 of *kat*G gene. No mutation in the both loci was detected in 3 (3.6 %) of INH^r isolates. Present study highlights the prevalence of common mutations associated with resistance in MDR *M. tuberculosis* strains spreading in Nepal, and also provides the basic information for developing molecular-based tests to detect MDR-TB in the clinical isolates.

Abstract

Poster Core time I at Lecture room 3 (Day 1: 16:30-17:30 on September 16)

P-3

Role of NF-kappa B signal inhibitor A20 and ABIN-3 in regression of TDM-induced tuberculoid granuloma in mice

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Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (MTB), is still one of the most important infectious diseases in the world. The hispathological feature of this disease is granuloma mainly consisted of epithelioid macrophages. Purpose of this study is to investigate the factors which is responsible for granuloma regression. For this purpose, trehalose-dimycolate(TDM), granulomagenic cell-wall glycolipid of MTB, was injected into mice and the granulomatous lesions were induced. From histopathological observation and mRNA expression levels of inflammatory cytokines, the regression phase of the lesions were determined. A20 and ABIN-3, negative feedback regulators of NF-kappa B signaling pathway, were demonstrated to be up-regulated in this granuloma regression phase. Thus, the correlation of NF-kappa B and A20-ABIN-3 in the formation and regression of the TDM-induced granulomatous was further investigated. The result demonstrated the increase of NF-kappa B in granuloma developping phase and the decrease after that. In addition, the level of NF-kappa B activation correlated with the size of the lesion. Next, protein levels of A20 and ABIN-3 in the lesion were quantified. The similar kinetics of the proteins to that of their mRNA levels was observed in a milder manner, i.e., high expression levels of A20 and ABIN-3 in regression phase. Finally, I tried to identify factors that interact with ABIN-3 by co-immunoprecipitation method. As a result, TAK-1 and A20 were immunoprecipitated with ABIN-3, demonstrating that TAK-1 and A20 interact with ABIN-3. Therefore, it can be possible that the ABIN-3 and A20-mediated suppression of TAK-1 induces the suppression of NF-kappa B, and this may lead to the regression of the granulomatous lesion.

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Poster Core time II at Lecture room 3 (Day 2: 16:15-17:15 on September 17)

P-4

An Analysis of Hospital-based Sentinel Surveillance Data on Leptospirosis in Sri Lanka, 2005 - 2008

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Leptospirosis, which is caused by spirochetes belonging to the genus Leptospira, is a worldwide public health problem. The occurrence of the disease is usually related with socio-economic and climatic conditions, which in turn favor animal vectors and human exposure. Disease surveillance is a critical component of the heath system in generating essential epidemiological information for a cost-effective healthcare delivery. Through surveillance, the incidence and distribution of diseases (e.g., leptospirosis) and their implications for effective public health strategies are identified. In Sri Lanka, leptospirosis is a notifiable disease first reported in 1953. In addition to a national notifiable disease reporting system, Sri Lanka has implemented a hospital-based sentinel surveillance system since 2004. This report discusses the findings of a descriptive analysis of sentinel surveillance data for 2005 – 2008, which was obtained from the Ministry of Health, Sri Lanka. Of 4000 suspected cases, approximately half (46.9%) and one fourth (26.8%) were recorded from Western and Sabaragamuwa Provinces, respectively. Most cases were male (83.5%) and approximately a half (45.6%) were aged 30-49 years. Farmers accounted for 16.5% and laborers for 16.1%; however, nearly a half (44.8%) had unknown occupations. Half (53.9%) had an exposure to paddy lands. Almost all had acute fever (98.8%), myalgia (92.9%) and headache (92.7%), but fewer had other related symptoms. Moreover, 2496 cases (62.4%) underwent a laboratory examination, and among the cases had laboratory investigation, only 1445 (57.9%) cases' laboratory test results were available at the sentinel sites and only 41 (2.8%) cases micro agglutination test results were known. Less than 2% of reported cases took an antibiotic prophylactic treatment. The study findings will help enhance the ongoing control and prevention efforts against leptospirosis in Sri Lanka. Sentinel surveillance is a useful tool, but needs to improve its data quality, and will greatly benefit if laboratory examination data adequately supplemented.

Abstract

Poster Core time I at Lecture room 3 (Day 1: 16:30-17:30 on September 16)

P-5

Genotying of *Ehrlichia ruminantium* by multiple-locus variable-number tandem-repeat analysis

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The rickettsial bacterium *Ehrlichia ruminantium* is the causative agent of heartwater, a serious tick-borne disease in ruminants. The genetic diversity of organisms in the field will have implications for cross-protection capacities of any vaccine developed, and for an effective vaccine design strategy proper genotyping and understanding of existing genetic diversity of field isolates is necessary. We searched for variable -number tandem-repeat (VNTR) loci for use in a multi-locus VNTR analysis (MLVA). Sequencing analysis of 30 potential VNTRs using a panel of 16 reference strains from geographically diverse origins identified 12 VNTRs with allelic profiles differing between strains. Application of MLVA to 38 *E. ruminantium*-infected *Amblyomma variegatum* collected from indigenous cattle in six different districts of Uganda identified 21 MLVA types. The discriminatory power of MLVA was greater than that of *map1* PCR-restriction fragment length polymorphism analysis, with which only 7 genotypes were obtained. In addition, since MLVA profiles from Ugandan field samples clustered separately from strains from other countries, this method may have potential for use in tracing the geographic origins of the bacteria as well as for monitoring genetic structure of *E. ruminantium* populations in the field.

Poster Core time II at Lecture room 2 (Day 2: 16:15-17:15 on September 17)

P-6

Whole genome next-generation sequencing reveals genome-wide nucleotide level polymorphisms in *Theileria parva*

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[Background] The disease caused by Apicomplexan protozoan parasite *Theileria parva* called East coast fever or Corridor disease, is one of the most serious cattle diseases in eastern, central and southern Africa. Genetic recombination between different lineages of the parasite during the sexual stage in the tick vector is considered to be the driver for the parasite diversification or evolution. Since the complete genome sequence of strain Muguga has been determined, genome-wide comparison is now possible at nucleotide level by using next generation sequencing technologies. A better understanding of the polymorphisms in this parasite may provide important information for studying parasite evolution, developing diagnostic and preventive methods.

[Material and methods] To reveal genome-wide polymorphisms, we performed whole genome re-sequencing of nine *T. parva* strains, including one of the vaccine strain (Kiambu 5), field isolates from Zambia, Uganda, Tanzania or Rwanda, and buffalo-derived strains. Schizonts from infected lymphocyte were purified by Percoll density gradation centrifugation, and extracted DNA was sequenced with the Illumina Genome Analyzer II (Solexa).

[Results] The short DNA reads obtained by Solexa were aligned to approximately 8.3 Mb of the *T. parva* Muguga genome sequence as a reference, and over the 90 % of the genome was re-constructed for each strain. Comparison with the reference Muguga genome sequence revealed 37,000-140,000 single nucleotide polymorphisms (SNPs), the highest number being in buffalo-derived strain. Phylogenetic analysis with the selected informative SNPs allowed the verification of the complicated recombination events among strains in the past. We further analyzed dN/dS ratio (non-synonymous substitutions per non-synonymous site) for 4,035 coding genes between Muguga and seven bovine-derived strains to estimate selective pressure. The genes under possible positive selections were selected, which may help us to explore immunogenic proteins or vaccine candidates.

Abstract

Poster Core time I at Lecture room 2 (Day 1: 16:30-17:30 on September 16)

P-7

Population genetic analysis and sub-structuring of *Theileria parva* in the northern and eastern parts of Zambia

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Theileriosis known as East Coast fever (ECF) is a very important economic disease in Africa caused by the protozoan haeamoparasite *Theileria parva* (*T. parva*). It is a major constraint to the development of the livestock industry in the eastern, central and southern parts of Africa. In Zambia, theileriosis causes losses of up to 10,000 cattle per annum and approximately 1.4 million of the 3 million cattle population is at risk to theileriosis. ECF is present in the northern and eastern provinces of the country while corridor disease (CD) is present in the southern and western provinces. In this study, we collected cattle blood samples for genetic analysis of *T. parva* from Isoka (n=91) and Petauke (n=46) districts of Zambia, approximately 600 km apart. All the samples were positive for *T. parva* by nested PCR screening using specific *T. parva* P104 gene primers. We analyzed the genetic structure of *T. parva* from a total of 60 samples (27 from Petauke and 33 from Isoka) collected from the two areas using a panel of 9 microsatellite markers encompassing the four chromosomes of *T. parva*. Population genetic analysis of the samples from the two areas showed a reduced gene flow and high level of genetic diversity in both Isoka and Petauke populations, implying absence of migration of parasites between the two sampling areas. Linkage disequilibrium and non-panmixia were observed when populations from both districts were treated as one population, but when analyzed separately, linkage equilibrium and panmixia was observed in the Petauke samples and linkage disequilibrium and non-panmixia in Isoka samples. Wright's F index showed moderate differentiation between the Petauke and Isoka populations. The population from Isoka was, however, more diverse than that from Petauke, suggesting that a subset of the Isoka population is responsible for the south-east ward spread of the disease into Petauke (Eastern Province).

Poster Core time II at Lecture room 2 (Day 2: 16:15-17:15 on September 17)

P-8

Development of a molecular diagnostic method for differentiation and quantification of *T. orientalis* Genotypes

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We report the development of diagnostic and quantifications of *Theileria orientalis* types (Ikeda, Buffeli and Chitose), the causative agent of theileriosis in cattle and its cohorts, using ITS1 and ITS2 spacers by fragment genotyping. We utilized primers flanking the two ribosomal RNA internal transcribed spacers (ITS1 and ITS2). Due to varying degrees of sequence polymorphism in the ITS regions found within and between species, we exploited the insertions and or deletions in these regions which resulted in different fragment sizes. On the basis of fragment size polymorphism, we were able to discriminate the three commonly found types of *Theileria orientalis.* ITS1 was capable of differentiating all three types (Ikeda-251bp, Chitose-274bp and Buffeli-269bp) in one single reaction by fragment genotyping. However, using ITS2, Ikeda (133-bp) a more pathogenic type was distinguishable from Buffeli/Chitose (139-bp). In addition, we quantified parasite load in experimental animals using ITS1. When compared with previous PCR detection method, ITS1 and ITS2 genotyping were found to be more sensitive methods with high specificity in population analysis and can be deployed in molecular epidemiology studies.

Abstract

Poster Core time I at Lecture room 2 (Day 1: 16:30-17:30 on September 16)

P-9

Molecular detection and sequence characterization of three novel piroplasm spp from wild felids in Kenya

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Most of Africa wild felid species can be found in Kenya. However, information on occurrence and molecular identity of piroplasm infections among these animals is lacking. Using a PCR assay targeting the 18S rRNA gene of all piroplasms, we screened blood samples that were collected from two leopards (*Panthera pardus*), five cheetahs (*Acinonyx jubatus*) and a pair of mildly anemic captive lions (*Panthare leo*) maintained in a zoo-park in Kenya. With the exception of three of the cheetahs, the rest of the animal samples were positive for piroplasm DNA. Genetic analyses with the near-complete 18S rRNA gene sequences obtained from these samples indicated the presence of two novel *babesia* parasites, one from the leopards and the other from the lions, and a *Theileria-like* sp. from the cheetahs. A neighbor-joining phylogenetic tree indicated the *Babesia* sp. parasite from the leopard was closely related to *Babesio leo* and *Babesia felis,* parasites that frequently infect lions and domestic cats, respectively. The cheetah *Theileria* sp. parasite identified from this study falls within the *Theileria sensu stricto* clade, indicating it is a true *Theileria*. These findings suggest greater diversity in piroplasms from African wild felids, and more piroplasms could yet be identified.

Poster Core time II at Lecture room 2 (Day 2: 16:15-17:15 on September 17)

P-10

Molecular epidemiological studies on Animal Trypanosomiases in Ghana

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African trypanosomes are extracellular protozoan parasites that are transmitted between mammalian hosts by the bite of an infected tsetse fly. Human African Trypanosomiasis (HAT) or sleeping sickness is caused by *T. brucei rhodesiense* or *T. brucei gambiense* while African Animal Trypanosomiasis (AAT) is caused mainly by *T. vivax*, *T. congolense* and *T. brucei brucei*. Trypanosomiasis is of public health importance in humans and is also the major constraint for livestock productivity in sub-Saharan African countries. There exists scanty information about the Trypanosomiasis status in Ghana especially regarding molecular epidemiology. Therefore, this study intends to apply molecular tools to identify and characterize trypanosomes in Ghana. A total of 250 tsetse flies, 248 pig and 146 cattle blood samples were collected from Adidome and Koforidua regions in Ghana in 2010. As a preliminary study, tsetse fly samples (n = 113) were screened for Trypanosoma infection by two PCR assays. Primer sets used in this experiment were Internal Transcribed Spacer 1 (ITS-1) primers, which can detect most of the pathogenic Trypanosome species, and *T. vivax* specific cathepsin L -like gene primers. The ITS-1 amplify a region conserved among Trypanosome species and give PCR products with species specific sizes, enabling species identification and multiplex detection of different species. The results indicated that twelve (10.6%) and ten (8.85%) flies were infected with *T. congolense* and *T. vivax*, respectively, while two (1.77%) were infected with both *T. congolense* and *T. vivax*. Our results showed that there is a high prevalence of parasites in tsetse flies in tested areas in Ghana. No *T. brucei* group was identified in our preliminary findings. Further analysis on the rest of the samples will enclose overall status of trypanosomiases in mammalian hosts and vector flies in Ghana.

Abstract

Poster Core time I at Lecture room 2 (Day 1: 16:30-17:30 on September 16)

P-11

Population genetic analysis of *Trypanosoma evansi* in the Sudan

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Trypanosomiasis due to *Trypanosoma evansi* is a widely distributed disease of livestock, affecting specially camelids and equines and is transmitted by biting flies. The disease is of great concern to many developing countries such as Sudan, where its large camel population estimated at over 4 million heads is at risk. It is generally believed that *T. evansi* has evolved when camels infected with *T. brucei* moved to tsetse free areas, and the parasite has recently been suggested to be considered as a subspecies of *T. brucei* complex. Nonetheless, only a few studies have been carried out to elucidate the genetic make-up of *T. evansi*. Therefore, in the current study, 15 microsatellite markers from non-coding loci on 38 isolates of *T. evansi* originating from different locations in Sudan were analyzed. Three reference strains from Sudan and Kenya were additionally analyzed and compared to the recent isolates. We detected a strong signal of isolation by distance across the area sampled. The results of this study suggested that *T. evansi* is either purely clonal structuring in small units at very local scales, or that it often sexually recombines without the need of the definitive host, the tsetse fly.

Poster Core time II at Lecture room 2 (Day 2: 16:15-17:15 on September 17)

P-12

Modeling Cutaneous Leishmaniasis Transmission in Costa Rica

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American Cutaneous Leishmaniasis (ACL) is one of the most important emerging and re-emerging neglected tropical diseases in the New World. Like many other vector-borne diseases there is a growing concern about the impacts of global climate change on its dynamics and spatial distribution. Cutaneous Leishmaniasis is also of special interest because of its diverse array of hosts. For example, ACL emergence has been directly or indirectly linked to changes in species diversity. Here, I will present results from studies on ACL in Costa Rica dealing with the internal relations of the disease with climatic variability and biodiversity changes. First, I will show a series of analyses describing the connections between the disease and global climate phenomena like El Niño Southern Oscillation, ENSO, and the time delays between exposure and clinical signs. Second, I will present a series of results on the relationship of the disease with biodiversity as inferred from mathematical models. These models showed that changes on the endemic equilibrium of ACL are only sensitive to changes in reservoirs species, and that, assuming a homogeneous host species use, increased biodiversity decreases the likelihood of disease establishment. Finally, I will describe ongoing research looking at the connection between climatic variability, land use change and vector and reservoir diversity on the spatial distribution of ACL in southern Costa Rica.

Abstract

Poster Core time I at Lecture room 2 (Day 1: 16:30-17:30 on September 16)

P-13

Reaction of complement factors varies with prion strains in vitro and in vivo

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Prion diseases are fatal neurodegenerative disorders in human and animals. Although previous studies reported that complement factors are involved in neuroinvasion of prion pathogen from the peripheral, roles of complement factors in prion infection of the central nervous system remain unclear. In this study, we attempted to assess possible involvement of complement factors in neuropathogenesis of prion disease. When neuro2a (N2a) cells persistently infected with either Chandler- and 22L-scrapie were cultured in the presence of normal mouse serum (NMS), the proportion of degenerating cells stained with the phosphatidylserine binding protein Annexin V were increased both in Chandler- and 22L-infected N2a cells. Preincubation of NMS with anti-complement C1q, C3 and/or C9 antibodies reduced Annexin V positive cells in Chandler-infected cells, while only anti-C3 antibodies were effective on 22L-infected cells. Membrane attack complex (MAC) was detected on Chandler-infected N2a cells, but not on 22L-infected cells. These results suggest that different complement component reacted with Chandler- and 22L-infected N2a cells. To assess potential differences in complement activation between these scrapie strains in vivo, we tested for C1q and C3 by immunohistochemistry in Chandler- and 22L-infected C57BL/6 mouse brains in the pre -clinical phase. Consistent with the reaction of complement factors to prion-infected N2a cells, C1q, but not C3, was detected in the vacuolar lesions of the dorsal part of thalamus in Chandler-infected mouse brains. In contrast, in the lesions of the same part of 22L-infected mouse brains, C3 was detected but C1q signals were lacking. These results suggest that the reaction of complement factors is different with prion strains in vivo as well as in N2a cells.

Poster Core time II at Lecture room 2 (Day 2: 16:15-17:15 on September 17)

P-14

Prion-infected neurospheres as ex vivo models for investigation of piron-induced neurodegeneration

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【Introduction】Prion diseases are progressive neuro-degenerative diseases that affect both human and animals. The mechanism of neurodegeneration caused by prion propagation still remains unclear. Neuroblastoma cells infected with prions have been used for studying the mechanism of prion propagation. However, neuroblastoma cells do not show apparent alteration by prion propagation, suggesting that they are inadequate for analyzing neurodegeneration caused by prion infection. Cell cultures that exhibit neuronal degeneration by prion propagation are sure to be useful for analyzing the mechanisms of neurodegeneration by prion infection, however, such *ex vivo* cultures are not available so far. To establish *ex vivo* experimental model in which neuronal degeneration by prion propagation can be reproduced, we prepared primary mouse neurospheres and characterized prion infection and neurodegeneration in differentiated neurospheres.

【Methods】Primary neurospheres were isolated from brains of 13-15 embryonic days of wild type mice. Neurospheres were differentiated by plating polyethyleneimine-coated plastic dishes or chamber slides and withdrawal of EGF and FGF2 from medium. Five to thirty days after differentiation, brain homogenates of mice infected with prion Chandler, 22L, Obihiro, G1, Fukuoka 1, or KUS strain were inoculated to differentiated neurospheres, and cultures were kept up to 80 days after inoculation. Cells were analyzed by double staining with mAb 132 for PrP^{Sc} and antibodies against markers for neurons, astrocytes, oligodendrocytes or NG2-positive cells. For the specific detection of PrP^{Sc} with mAb 132, cells fixed with paraformaldehyde were treated with guanidinium thiocyanate prior to immunostaining.

【Results】The differentiated neurospheres became positive for PrP^{Sc} by the inoculation of 6 different prion strains, suggesting that primary neural cultures from neurospheres are susceptible to many different type of prions. The differentiated neurospheres could be maintained over 80 days after inoculation. PrP^{Sc} was preferentially detected in GFAP-positive astrocytes up to 20 days after inoculation, in contrast, a few PrP^{Sc} -positive neurons became detected at 20 days after infection and gradually increased thereafter.

【Discussion】The differentiated neurospheres expressed several pre- and post- synaptic markers such as SNAP25 and PSD95. Thus, we are now planning to assess the neurodegeneration by quantitative analysis of synaptic markers, annexin V reactivity and TUNEL staining. Further characterization will reveal the utility of this *ex vivo* cultures for the analysis of neurodegeneration caused by prion infection.

Abstract

Poster Core time I at Lecture room 2 (Day 1: 16:30-17:30 on September 16)

P-15

Surveillance of Marek's disease virus in migratory and sedentary birds in Hokkaido, Japan

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Marek's disease (MD) is a lymphoproliferative disease of chickens caused by a cell-associated alphaherpesvirus, Marek's disease virus (MDV). MDV is classified into 3 serotypes (serotype 1, 2, and 3), and MDV serotype 1 strains, other than attenuated vaccine strains, cause MD. Currently, MD is well controlled by the administration of live vaccines. However, MDV tends to increase in virulence, and several MD cases in vaccinated chickens have been reported, suggesting that highly virulent MDV could potentially induce future outbreaks. In addition, in 2001, an MD case was reported in a white-fronted goose (*Anser albifrons*) that had migrated from Russia to Hokkaido, Japan. Previously, we investigated the prevalence of MDV in wild waterfowl and ascertained that MDV is widespread in white-fronted geese. Because wild waterfowl usually flock together and migrate between breeding habitats and warm areas, they could play a role as reservoirs and carriers of MDV to domestic poultry. Although MDV is widespread in geese, clinical MD cases, except for that in 2001, have not been reported; therefore, most wild geese may have resistance to MD. Furthermore, MDV may be prevalent in other wild birds, in which MD remains asymptomatic. In this study, we investigated the prevalence of MDV in wild birds by using nested PCR to screen for the *meq* gene in DNA samples extracted from feather tips. Samples were collected from 309 wild geese and ducks and 40 other wild birds in Hokkaido. The *meq* gene was detected in a high percentage of geese and ducks, whereas other birds tested negative, suggesting that MDV is widespread in wild goose and duck populations, but is not prevalent in other birds. The *meq* gene product, Meq, which is a putative oncoprotein of MDV, contributes to oncogenicity by altering the expression of various cellular genes. Therefore, changes in the structure of the Meq protein may alter transactivation activity, indicating that diversity in Meq may alter its function and subsequently contribute to oncogenicity. Consequently, we established the amino acid sequences in the Meq proteins of the detected *meq* genes, and distinct diversity was found in the Meq proteins of the waterfowl samples. The amino acid substitution at 77 in the Meq proteins has been identified in most of the virulent MDV strains, and in samples of mallards, pintails, and spot-billed ducks, this substitution was identified, suggesting that highly virulent MDV strains may be prevalent in wild ducks. Some wild duck populations, in which the *meq* gene was detected, are resident in Japan, indicating that MDV occurs not only in migratory, but also in sedentary waterfowl; therefore, resident ducks could be carriers and reservoirs, regardless of season. To control field outbreaks of MD in the future, periodic monitoring of MDV in wild waterfowl is advisable.

Poster Core time II at Lecture room 2 (Day 2: 16:15-17:15 on September 17)

P-16

The identification of the rodent reservoir of Montano virus, a novel hantavirus in Mexico

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Hantaviruses are important causative agents of hantavirus pulmonary syndrome (HPS) in humans with about 40% fatality. Our earlier survey in Guerrero and Morelos States of Mexico indicated that several distinct hantaviruses may be circulating in wild rodents. To broaden our knowledge on the epidemiology of hantavirus infections in Mexico, the S gene of a newly identified Mexican hantavirus (Montano virus; MTNV) was cloned and recombinant nucleocapsid protein (rNP) was expressed. Two hundred wild rodent sera from Guerrero and Morelos States were analyzed by ELISA using this antigen. The MTNrNP detected 14 (7%) hantavirus seropositives as did the Sin Nombre virus recombinant nucleocapsid protein (SNrNP) antigen. ELISA results were confirmed by Western blotting (WB) and also by the detection of hantavirus S gene by RT-PCR in the lung tissues of the majority of antibody positive rodents. The open reading frame of S gene was sequenced. Morphological species identification among Mexican rodents was quite difficult. Therefore mitochondrial DNA cytochrome b of hantavirus positive rodents was sequenced. Phylogenetic analysis showed that MTNV is genetically distinct from other North American viruses and *Peromyscus beatae* serving as the main carrier of the virus. Serology results by MTN- and SNrNP antigens were completely matched, suggesting that both antigens are useful for detecting hantavirus antibodies. In the absence of live virus, monoclonal antibodies to MTNrNP were prepared to evaluate the antigenic relationships and also for differential detection of Mexican with other hantaviruses. The majority of the mAbs were still broadly cross reactive within North American hantaviruses and binding studies showed that most of the mAbs were confined to the N-terminal amino acid residues 1-51.

Abstract

Poster Core time I at Lecture room 2 (Day 1: 16:30-17:30 on September 16)

P-17

Development of immunochromatography strip-test for detecting of anti-hantavirus antibody in human and rat sera

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[Background] Hantavirus is a genus of rodent-borne trisegmented three segment negative-strand RNA viruses in the family Bunyaviridae. Various species of hantaviruses are broadly distributed all over the World. New World Hantaviruses cause a hantavirus pulmonary syndrome (HPS) in the Americas, whereas Old World hantaviruses cause a hemorrhagic fever with renal syndrome (HFRS) in Europe and Asia. To detect Hantavirus infection, PCR and ELISA were developped. However, these analyses were required laboratory equippments and tools and time-consuming tests. Therefore, it is needed to develop a simple, rapid, and accurate method for the detection of Hantavirus infection. In this study we tried to develop an immunochromatography (ICG).

[Materials and Methods] The ICG technique based on a strip of paper coated with an immobilized antibody specific for an antigen that is characteristic of a disease.For the developpment of ICG for human sera, Protein A Colloidal Gold (EY LABORATORIES) was used as capture antibody in the conjugate pad. Conjugate-antibody complex flow on the membrane and the complex is caught by antigen. N-ternimal 103 amino acids of hantaviral N proteins were expressed in E. coli by using pET43.1 vector (Novagen) and purified by His-Trap column (GE). Normal human serum was used as control line. To detect anti-hantavirus antibody in rat sera, the conjugate was prepared by using Colloidal Gold WRGH2 (Wine red Chemicals) and rabbit anti-rat IgG protein (Sigma). To evaluate the ICG strip-test, two kinds of HFRS patient sera caused by Hantaan virus (HTNV) and Seoul virus (SEOV) were used. In addition to the patient sera, rats sera infected with HTNV or SEOV infected with laboratory and wild were used. To evaluated ICG methods, healthy human sera and anti-hantavirus antibody negative rat sera were used.

[Results and Discussion] Sera from HTNV and SEOV infected patients were diluted with PBS and then applied on conjugation pad. Infected patient sera showed both test and control line signals and uninfected human serum showed only control line signals. Similarly, antibody positive rat sera showed test-lines. This ICG strip-test could be applied to both HTNV and SEOV infection because of their cross-reactivities. By using diluted sera, reactivity in ICG was almost same as those of ELISA. Sensitivities and specificities of ICG tests and rat sera 86%, 100% , respectively. Multiplex ICG to diagnose both HPS and HFRS are on proceeding. In future, applicability of ICG will be examined by using huge l numbers of human and rodent sera.

Poster Core time II at Lecture room 2 (Day 2: 16:15-17:15 on September 17)

P-18

Cytotoxic T lymphocyte (CTL) responses to hantavirus in laboratory and wild rats

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Seoul virus (SEOV), one of the serotypes of hantavirus, is a causative agent of hemorrhagic fever with renal syndrome. SEOV is maintained in *Rattus norvegicus* with persistent infection. However, the mechanism by which causing persistent infection is still unclear. To examine the relationships between hantavirus infection and suppression of cytotoxic T lymphocytes (CTLs), we attempted to establish methods for immunological studies using transiently infected laboratory rats, a laboratory strain of *R. norvegicus*.

Rats (WKAH, male, 6 weeks old) were experimentally infected with SEOV strain SR-11 (6.0×10^4 ffu/rat, intraperitoneal route). Lungs, sera and splenocytes were collected at periodic intervals. IgM and IgG antibody titers were measured using the ELISA method. The amount of virus was examined using a real-time PCR method. The mitogenic responses of splenocytes were evaluated using concanavalin A (ConA response). Hantavirus specific CTLs were detected using ELISPOT assay with 309 kinds of 15-amino acid peptides as epitopes which covered entire glycoprotein and nucleocapsid protein sequences.

IgM antibody appeared at 6 days post-inoculation (dpi) and IgG antibody appeared at 9 dpi. The IgM antibody titers began to decrease at 13 dpi, whereas the IgG antibody titers increased continuously. The amount of virus peaked at 3 dpi and then decreased in lung and disappeared in serum. ConA response of splenocytes from infected rats showed same level to those from non-infected ones. ELISPOT assay defined WKAH strain specific three epitopes. CTLs were activated stronger by epitope #149, located in glycoprotein Gc, than others. ELISPOT assay using epitope #149 showed that CTLs activation were peaked at 6 dpi.

Thirty-four wild *R. norvegicus* were collected in HaiPhong Port, Vietnam in Jan 2011. Sixteen of 34 individuals were hantavirus-positives detected by IgG and IgM ELISA. ConA responses of splenocytes were compared between infected and non-infected rats. ConA responses of splenocytes from infected rats were a bit higher than those from non-infected rats, though no significant differences ($p = 0.055$) were detected. Compared to experimentally infected rats, ConA responses of infected and non-infected wild rats were low.

These results show that the ConA response and the ELISPOT assay are applicable for immunological study in SEOV infected rat. Further study for apply these methods to naturally infected *R. norvegicus* will be necessary to examine mechanisms for persistent infection in the natural host.

Abstract

Poster Core time I at Lecture room 2 (Day 1: 16:30-17:30 on September 16)

P-19

Analysis of pulmonary edema in hantavirus-infected SCID mouse

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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. Acute respiratory distress syndrome (ARDS) with pulmonary edema has been reported in severe cases of both HFRS and HPS patients.

We have developed a mouse pulmonary edema model by adoptive transfer of immune splenocytes into Hantaan virus (HTNV)-infected SCID mice. The mice showed severe lung edema of which wet lung weight at 6 days after transfer was more than 2-fold greater than that of mock and histological change was similar to that in HPS patient.

In this study, we examined cell populations in bronchoalveolar lavage fluid (BALF) and lung homogenate (LH) of mice with lung edema at 6 days after transfer by morphological analyses and FACS.

Total number of cells in BALF of the model was markedly increased (3-fold) compared with that in controls. Lymphocytes constituted the major population in BALF (approx. 75%). The majority of lymphocytes were T-lymphocytes (approx. 30%) and the percentage of B-lymphocytes was relatively small (approx. <5%). The number of neutrophils was also increased. The number of macrophages was similar to that in controls. Likewise, the percentage of T-lymphocytes was higher than that of B-lymphocytes in the LH. Neutrophils were prominently increased to approx. 40% (control <20%). The percentages of macrophage and dendritic cells were similar to those in controls. These results indicate that T-lymphocytes and neutrophils might have an important role in inducing lung edema.

Since neutrophils play a key role in the physiopathogenesis of general ARDS, the mouse model might mimic human ARDS caused by hantavirus. Further studies, such as studies in which each lymphocyte subset alone is transferred and neutrophils are depleted, are needed to determine the mechanism of pulmonary edema in hantavirus infection.

Poster Core time II at Lecture room 2 (Day 2: 16:15-17:15 on September 17)

P-20

Development of the lethal animal model of human hantavirus infection

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[Introduction] Hantaviruses are the causative agents of hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS) in humans. Both HFRS and HPS are characterized enhanced capillary leakage. Hantaan virus (HTNV) is one of the causative agents of HFRS in East Asia. Although the experimental animal model of HTNV infection was developed using suckling mice, infected animals die in encephalitis, which is completely different from the pathology of HFRS. Thus it is required more suitable model to analyze the pathogenesis of HFRS. When HTNV strain Galkino/AA57/2005 (AA57) was inoculated to ICR mice, some mice suddenly weakened died. Thus we analyzed the pathological changes of AA57-infected mice and investigated whether this model was the suitable model for HFRS.

[Materials and Methods] Two weeks old ICR mice were inoculated with AA57 subcutaneously. Mice were observed for 3 weeks and organs of dead mice were collected for virus titration and pathological analysis.

[Results] Mice inoculated with AA57 began to have some clinical signs (body weight loss, labored breathing) at 8-11 dpi and some mice died until almost 1 day after onset. Morbidity was 10-70% (3-30000ffu/head) and mortality was 20-30% (30-30000ffu/head). A large amount of pleural effusion (2.7% of body weight) was collected from all dead mice (12/12). The highest titer (1733ffu/gram) of AA57 were detected from lung (11/12) and lower titer (533ffu/gram) of this virus were detected from spleen (5/12), kidney (1/12), heart (3/12) and brain (3/8). The reduction of alveolar space with the thickened interstitium and overgrowth of mononuclear cells were observed in dead mice (3/3). Hemorrhage (2/3) and defluxion of mononuclear cells (3/3) into alveolus were observed in these mice.

[Conclusion] Although these pathological findings were rather similar to HPS than HFRS, this is the first observation that HFRS-related hantavirus may enhance capillary leakage in mice.

Abstract

Poster Core time I at Lecture room 2 (Day 1: 16:30-17:30 on September 16)

P-21

Isolation of Hokkaido virus cultured cells derived from the kidney of gray red-backed vole (*Myodes rufocanus*)

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[Introduction] Hantaviruses belong to the *Bunyaviridae* family and cause two severe human illnesses, hemorrhagic fever with renal syndrome (HFRS) and hantavirus cardiopulmonary syndrome (HCPS). More than 30 viruses have been reported in the genus *Hantavirus* and are carried by the specific rodent host. Although VeroE6 cells originating from African green monkey kidney are widely used for hantavirus research, growth and isolation of hantaviruses in this cell line is very slow and difficult. To develop efficient method to isolate hantavirus, we established new cultured cells derived from a kidney of gray red-backed vole (*Myodes rufocanus*), the natural host of Hokkaido virus (HOKV). We used this cultured cells to isolate HOKV.

[Materials and Methods] In 2010, a total of 82 *M. rufocanus* were captured in Tobetsu town, Hokkaido, Japan. Kidneys from 7 *M. rufocanus* were trypsinized, seeded into 25 cm^2 flasks, and cultured in Dulbecco's modified minimum essential medium with 10% fetal bovine serum. One of these seven cell cultures had good multiplication and has been continuously subcultured for more than 7 months. The name of cultured cells was designated as MRK101 cell. To isolate HOKV, lung and kidney tissues from HOKV infected *M. rufocanus* were inoculated into suckling Syrian hamsters. Fifteen days postinoclation (dpi), lung and kidney tissues were collected from hamsters. The supernatant of tissue homogenates was inoculated to both MRK101 cells and VeroE6 cells. The virus RNA and antigen in the inoculated cells were detected by RT-PCR and IFA, respectively.

[Results] In inoculated VeroE6 cells, the viral RNA was detected from 14 dpi but viral antigen has not been detected even 2 months postinoculation. In contrast, viral RNA and antigen were detected from 14 dpi in inoculated MRK101 cells. The presence of infectious virus in culture fluid of inoculated MRK101 cells was confirmed by inoculation to naïve MRK101 cells. Therefore, HOKV was successfully isolated.

[Discussion] It is generally known that hantavirus isolation is difficult and often fails. Even in successful cases, more than 2 months are required to isolate hantaviruses in VeroE6 cells. All attempts to isolate HOKV have been failed for more than 15 years. By using MRK101 cells, however, HOKV was successfully isolated in a short period. Therefore, MRK 101 cells may be susceptible to hantaviruses. Inoculation of tissue homogenates from infected rodents or patient samples to MRK101 cells could be a solution to isolate hantaviruses.

Poster Core time II at Lecture room 2 (Day 2: 16:15-17:15 on September 17)

P-22

Role of hantavirus nucleocapsid protein in intracellular traffic of glycoproteins

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Hantaviruses belonging to the family *Bunyavridae* are important rodent-born zoonotic pathogens causing hemorrhagic fever with renal syndrome and hantavirus pulmonary syndrome. The genome consists of tri-segmented negative-strand RNA encoding nucleocapsid protein (N), glycoproteins Gn and Gc, and RNA-dependent RNA polymerase. The fact that hantaviruses don't have matrix protein raises the hypothesis that N has a key role in assembly of viral components. In this study, to understand the role of N in viral assembly, the effect of N on intracellular traffic of glycoproteins was investigated. Double staining of viral and host proteins showed that Gn and Gc were localized to cis-Golgi in Hantaan virus (HTNV)-infected Vero E6 cells. When Gn and Gc were expressed by plasmid vector, Gn was localized to cis-Golgi, whereas Gc was dispersed into cytoplasm in about 30% of Gc-positive cells. The rate of the diffused Gc-positive cells significantly decreased by co-expression of N. N of other hantaviruses, such as Seoul virus, Puumala virus and Sin nombre virus, also reduced the rate of diffused Gc-positive cells heterologously. Analysis of mutant N and Gc showed that amino acid positions from 1 to 30 and 116 to 155 of N and Gc cytoplasmic tail region were important for the phenomenon. These results suggest that hantavirus N promotes cis-Golgi localization of Gc.

Abstract

Poster Core time I at Lecture room 2 (Day 1: 16:30-17:30 on September 16)

P-23

Construction and Characterization of Chimeric Virus between Tick-Borne Encephalitis Virus and Omsk Hemorrhagic Fever Virus

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Background:
Most of mammalian tick-borne flavivirus, such as Tick-borne Encephalitis virus (TBEV), causes encephalitic disease, while Omsk Hemorrhagic Fever virus (OHFV) is known to cause hemorrhagic disease. Recent phylogenetic analysis revealed that OHFV falls within the tick-borne encephalitis serocomplex of flavivirus and is distinct from other hemorrhagic tick-borne flaviviruses. The detailed mechanism that causes the differences between encephalitic and hemorrhagic disease still remains unclear in tick-borne flaviviruses. In this study, chimeric viruses between TBEV and OHFV were generated, and characterized to indentify the viral molecular determinant involved in the difference of the pathogenicity.

Methods:
By using infectious cDNA clone of TBEV Oshima strain and OHFV Guriev strain, we generated chimeric viruses whose genes for viral proteins were replaced with each other. BALB/c mice were infected with the recombinant viruses, and the animals were observed for signs of illness. The viral multiplication in organs and histopathologic features were analyzed.

Results:
Most of the mice infected with the virus with TBEV-C and NS proteins showed neurologic signs of illness while few mice infected with the virus with OHFV-C and NS proteins showed the signs. Early viral multiplication in brain was observed by the infection of the virus with TBEV-C and NS proteins. Although viral antigens were observed in the neuronal cells in the mice infected with each virus, the virus with TBEV-C and NS proteins caused more severe encephalitis. Apoptosis induction in neuronal cells was observed by the infection of the virus with TBEV-C and NS proteins.

Conclusion:
Our results indicated that C or NS proteins of TBEV are molecular determinants which cause encephalitic diseases. It was also suggested that the apoptotic induction in neuronal cells was involved in the neurological symptoms caused by the TBEV infection. This study will contribute to further understanding of the pathogenicity of tick-borne flaviviruses.

Poster Core time II at Lecture room 2 (Day 2: 16:15-17:15 on September 17)

P-24

Steric shielding of host proteins by filovirus glycoproteins

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[Background] Ebola and Marburg viruses are enveloped, negative-stranded RNA viruses and constitute the family *Filoviridae*. Filovirus infection causes severe hemorrhagic fever in human and non-human primates and mortality rates have ranged up to 90%. Different pathogenicity has been observed among filovirus species or strains. Despite extensive research, the molecular basis for the pathogenicity of filovirus remains elusive.

The filovirus glycoprotein (GP) contains a mucin-like region (MLR) which is highly glycosylated and occupies spatially very large region. Previous reports showed that MLR formed a steric shield over host proteins on the surface of GP-expressing cells and might abrogate cell adhesion and prevent interaction between lymphocytes and infected cells. Therefore, it has been suggested that the steric shielding effect is associate with the pathogenesis of filovirus infection.

[Purpose] In this study, to know possible contribution of the steric shielding to the pathogenicity of filovirus, we compared the shielding effects on cell surface host protein presentation among filovirus species or strains that show different pathogenicity. In addition, we analyzed whether the steric shielding effect is affected by GP cleavage by host ubiquitous protease, furin.

[Materials and methods] Following transfection of HEK293T cells with plasmid encoding wild type Ebola GP, Marburg GP, or uncleaved their mutants produced by modification of the furin cleavage sites, integrin β1 and MHC class I molecules expressed on cell surface were stained with fluorochrome-conjugated antibodies and analyzed with flow cytometry. When the steric shielding occurred, expression levels of these host proteins were expected to be apparently decreased in flow cytometry.

[Results] GP of *Zaire ebolavirus*, which caused multiple outbreaks with the highest mortality rates in humans among Ebola virus, masked integrin β1 markedly compared with that of *Reston ebolavirus*. Furthermore, GP of Marburg virus Angola strain, which caused the largest outbreak with the highest mortality rate among Marburg virus strains, shielded the integrin β1 more significantly than GP of Marburg virus Musoke strain. While MHC class I molecules on cells expressing wild type GPs of Ebola or Marburg viruses was almost undetectable, the shielding effect by uncleaved GP of Musoke strain was less prominent than wild type GPs. As expected, we found reduced shielding effects in cells expressing MLR-deleted GP.

[Discussion] The efficiency of steric shielding of integrin β1 was correlated with the pathogenicity of filoviruses. The difference of steric shielding effects observed among filoviruses was likely due at least in part, to the difference of amino acid sequence and glycan structure in MLR of GPs. The reduced shielding of MHC class I molecules by uncleaved Musoke GP suggests that the GP cleavage results in alteration of the structure and/or flexibility of the GP molecule, which is required for optimal effects of steric shielding.

Abstract

Poster Core time I at Lecture room 2 (Day 1: 16:30-17:30 on September 16)

P-25

Functional role of TIMP-1 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 critical step of host defense response. When a host organism is infected with viruses or bacteria, an inflammatory response occurs within the organism. After the infection, various types of inflammatory cells such as neutrophils, macrophages and lymphocytes infiltrate into the infected area from the blood. However, to reach the infected site, these cells have to run through various barriers, such as a blood vessel basement membrane. 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 functional role of these molecules (ECMs, cell adhesion molecules, MMPs and MMP inhibitors) after the viral infection is still unclear.

In this study, we found alterations of gene expression of several MMPs and ECMs in the lungs of mice infected with influenza virus A/Puerto Rico/8/34 (A/PR/8) (H1N1). In particular, the mRNA expression of tissue inhibitor of metalloproteinase-1 (TIMP-1), which is a specific inhibitor of MMPs, was remarkably induced in the lung after PR/8 virus infection. Therefore, we focused on the function of TIMP-1 for the pathogenesis by PR/8 virus infection, using TIMP-1 knockout (KO) and wild type (WT) mice. After the viral infection, the survival rate of TIMP-1 KO mice was lower than that of WT mice. In addition, at 7 day after infection, hemorrhagic tendency was detected in the bronchoalveolar lavage fluid (BALF) in TIMP-1 KO mice, and inflammatory cell accumulation in BALF was increased in TIMP-1 KO mice, compared with WT mice. These results suggest that TIMP-1 is involved in the regulation of vascular permeability in the lung inflammatory legion during PR/8 virus infection, and protects lung tissue from excessive inflammatory response such as hyperemia, exudation, edema after influenza virus infection. To address the pathophysiological role of TIMP-1, analysis of the expression and activity of TIMP-1 in the tissues is currently in progress.

Poster Core time II at Lecture room 2 (Day 2: 16:15-17:15 on September 17)

P-26

An H5N1 highly pathogenic avian influenza virus that invaded Japan through waterfowl migration

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Since 1996, highly pathogenic avian influenza virus (HPAIV) of H5N1 subtype have been circulating in poultry for more than a decade and causing unprecedented outbreaks in wild birds and poultry in Asia, the Middle East and Africa. Wild waterfowl, which are natural reservoir hosts of influenza A viruses, have been suspected to act as long-distance vectors and disseminators of H5N1 HPAIVs. During active surveillance of avian influenza in October 2010, an H5N1 HPAIV was isolated from fecal samples of apparently healthy migratory ducks having brief stops during their southward migration at Lake Onuma in Wakkanai, Hokkaido, the northernmost prefecture of Japan. H5N1 HPAIVs closely related to the Hokkaido isolate were subsequently detected in domestic and wild birds at multiple distinct sites corresponding to the flyway of the waterfowl in the Japanese archipelago. We found that the Hokkaido isolate was genetically nearly identical to H5N1 HPAIVs isolated from swans in the spring of 2009 and 2010 in Mongolia, as indicated by 98.9-99.8% nucleotide sequence identity between the Hokkaido and 2009 Mongolian isolates. It was noted that the Hokkaido isolate was less pathogenic in experimentally infected ducks than the 2009 Mongolian isolate. Five of the eight ducks infected with the 2009 Mongolian isolate died at 4-8 days post-infection. By contrast, all ducks infected with the Hokkaido isolate were nearly asymptomatic. These findings suggest that H5N1 HPAIVs with relatively mild pathogenicity might be selected and harbored in the waterfowl population during the 2009-2010 migration seasons. Our data provide "early warning" signals for preparedness against the unprecedented situation in which the waterfowl reservoirs serve as perpetual sources and disseminators of HPAIVs.

Abstract

Poster Core time I at Lecture room 2 (Day 1: 16:30-17:30 on September 16)

P-27

Characterization of avian influenza viruses isolated from domestic ducks in Vietnam in 2009 and 2010

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In the surveillance of avian influenza in Vietnam in 2009-2011, 1 H3N2, 1 H3N8, 7 H4N6, 1 H5N1, 31 H6N2, 1 H6N6, 26 H9N2, 1 H9N6, 3 H11N3, 1 H11N5, 3 H11N9, and 2 H12N5 viruses were isolated from trachea and cloaca swab samples of 1,300 domestic ducks and 328 wild birds.

Out of the 27 H9 virus isolates, the hemagglutinins of 18 strains were genetically classified into G1 and the other 9 into Korean sublineages. Phylogenetic analysis revealed that one of the 27 H9 viruses was a reassortant, in which the PB2 gene belonged to Korean sublineage and the other 7 to G1 sublineage.

Three representative H9N2 viruses were intranasally inoculated into ducks, chickens, pigs, and mice. On the basis of experimental infection studies, it was found that each of 3 viruses readily infected pigs and replicated in their upper respiratory tracts, and infected chickens with slight replication. Viruses were recovered from the lungs of mice inoculated with 2 of the 3 isolates.

The present results reveal that H9 avian influenza viruses are prevailing and genetic reassortment occurs among domestic ducks in Vietnam. It is recommended that careful surveillance of swine influenza with H9 viruses should be performed to prepare for pandemic influenza.

One H5N1 virus isolated from a duck in 2011 indicated that the intravenous pathogenicity index (IVPI) against chickens was 3.0. Phylogenetic analysis revealed that the HA gene of the H5N1 virus belonged to clade 1 and it was different from the clade 2.3.2 of the HA of the H5N1 viruses occurred in Hokkaido in the end of 2010-spring 2011.

Poster Core time II at Lecture room 2 (Day 2: 16:15-17:15 on September 17)

P-28

Comparison of potency of whole virus particle and ether split pandemic influenza vaccine prepared from A/swine/ Hokkaido/2/1981 (H1N1)

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The 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 feral and domestic water birds and pigs have played roles in the emergence of pandemic influenza viruses. In the present study, influenza virus A/swine/Hokkaido/2/1981 (H1N1) (Sw/Hok/81) was selected as the A/2009 (H1N1) pandemic influenza vaccine strain on the basis of its antigenicity and growth capacity in chicken embryos. Potency of 2 types of test vaccines, inactivated whole virus vaccine and ether split vaccine, was assessed by virus challenge test in mice.

BALB/c mice were injected subcutaneously either once or twice at a 2-week interval with inactivated whole virus vaccine or ether split vaccine prepared from Sw/Hok/81. Three weeks after the injection, the mice were challenged with a pandemic strain, A/Narita/1/2009 (H1N1) (Narita/09).

Mice injected once with inactivated whole virus vaccine prepared from Sw/Hok/81 developed cross-protective immune responses and had milder symptoms of infection with Narita/09 than the mice with ether split vaccine. Mice injected twice with ether split vaccine showed similar levels of protective efficacy to those inoculated once with inactivated whole virus vaccine. The present results suggest that whole inactivated vaccine prepared from influenza virus isolates from natural host in the influenza library in our laboratory was ideal vaccine strain on the early stage of future pandemic influenza.

Abstract

Poster Core time I at Lecture room 2 (Day 1: 16:30-17:30 on September 16)

P-29

Potency of an inactivated avian influenza vaccine prepared from a non-pathogenic H5N1 virus against the challenge with an antigenically drifted highly pathogenic avian influenza virus of clade 2.3.4

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H5N1 highly pathogenic avian influenza virus (HPAIV) infection has become endemic in 4 countries; China, Vietnam, Indonesia, and Egypt, where vaccine is used as a tool for the control of HPAI. As the reason why antigenically drifted viruses have been isolated from domesitic birds in Asia, it is assumed that these antigenic variants have been selected under the antibody pressure induced by vaccination in domestic bird population.

In the present study, vaccine prepared from a non-pathogenic H5N1 avian influenza virus from the library in our laboratory was assessed its potency to induce protective immunity against the challenge with HPAIV belonging to the clade 2.3.4 in chickens.

Antigenic analysis revealed that a HPAIV, A/peregrine falcon/810/2009 (H5N1) belonging to the clade 2.3.4, is antigenically different from those of clade 2.2, 2.5, and 2.3.2. The vaccine, oil-adjuvanted inactivated whole virus particles of A/duck/Hokkaido/Vac-3/2007 (H5N1), was inoculated into thigh muscle of four-week-old chickens. Three weeks later, these birds were challenged with A/peregrine falcon/Hong Kong/810/2009 (H5N1).

Although vaccinated chickens shed viruses much lower titers compared with those from unvaccinated chickens after challenge, two out of 12 chickens died on 4 and 7 post-challenge days and remaining 10 chickens survived.

The present results indicate that it is required to prepare vaccine from viruses antigenically similar to these variants prevailing domestic poultry population. However, if vaccine is used for the control of avian influenza, it is obvious that antigenic variants should be selected. We must, therefore, urgently eradicate viruses prevailing in domestic poultry in Asia by stamping-out policy without misuse of vaccine.

Poster Core time II at Lecture room 2 (Day 2: 16:15-17:15 on September 17)

P-30

Rapid replication of H7 highly pathogenic avian influenza virus induces hyper expression of cytokine mRNA in chickens

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Outbreaks of highly pathogenic avian influenza have causes enormous economic loss in poultry industry and public health threat over the world. The reason why chickens suddenly die due to infection with highly pathogenic avian influenza viruses (HPAIVs) is not well 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). To better understand the basis of the pathogenicity of these HPAIVs in chickens, $10^{6.0}$ EID_{50} of each strain was inoculated intranasally into chickens and compared viral growth and host immune responses in early stage of infection.

All of the chickens intranasally infected with $10^{6.0}$ EID_{50} of Ty/Italy/99 died in 3-4 days, while a half of the birds infected with the same dose of Ck/NL/03 died in 6-7 days and the others recovered. In growth curves of these viruses in chickens after inoculation, Ty/Italy/99 replicated efficiently in each tissue of the chickens, especially in the brain, at the early stage of infection. On the other hand, Ck/NL/03 replicated significantly more slowly than Ty/Italy/99 in each tissue of the infected chickens. The viral titers in the blood of the chickens infected with Ty/Italy/99 rapidly increased, while in the chickens infected with Ck/NL/03 lower titers of virus were detected. In the real-time PCR analysis, brisk expressions mRNAs of IFN-α, IL-1β, IL-6 and IFN-γ in each tissue of the chickens infected with Ty/Italy/99 increased in corelation with extensive replication of virus. In contrast, the cytokine mRNA expression in the chickens infected with Ck/NL/03 was less extensive.

The present results indicate that rapid replication of virus induces hyper expression of cytokine mRNA, leading cytokine storm and multiple organ disorder followed by sudden death of chickens. To assess the importance of the cytokine strom in the sudden death of the chickens infected with HPAIVs, the relationship of the viral growth and host immune responses in the chickens inoculated with low pathogenic avian influenza virus is under investigation.

Abstract

Poster Core time I at Lecture room 2 (Day 1: 16:30-17:30 on September 16)

P-31

Cross-reactibity of HA-specific antibodies induced by immunization with inactivated influenza viruses in mice

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Influenza A viruses are divided into subtypes based on the antigenicity of two envelope glycoproteins, hemagglutinin (HA; H1-H16) and neuraminidase (NA; N1-N9). It is well documented that HA-specific neutralizing antibodies play an important role in acquired immunity against influenza virus infection. The HA subtypes of influenza A viruses are principally defined as serotypes determined by neutralization or hemagglutination inhibition tests using polyclonal antisera to the respective HA subtypes, which have little cross-reactivity to the other HA subtypes. Inactivated influenza vaccines can efficiently induce antibody responses against influenza virus infection, which is, however, specific for antigenically related strains. Thus, it is desirable to develop new strategies which induce broadly cross-protective immunity against influenza viruses of multiple subtypes.

Previous studies showed that intranasal immunization with inactivated influenza viruses induced B-cell-dependent heterosubtypic immunity in mice. Previous reports demonstrated HA-specific monoclonal antibodies capable of neutralizing several influenza viruses of multiple subtypes. These studies suggested that cross-reactive antibodies play a role in heterosubtypic immunity against influenza virus infection. Therefore, in this study, antibodies induced by immunization of mice with inactivated viruses were investigated for their cross-reactivity to multiple HA subtypes.

Serum, lung wash (LW), and nasal wash (NW) samples were collected from mice immunized three times intranasally or subcutaneously with inactivated H1, H3, H5, H7, H9, or H13 viruses, and analyzed for the cross-binding and neutralizing activities of antibodies by enzyme-linked immunosorbent assay (ELISA) using recombinant HAs as antigens and plaque reduction assay. Both IgG and IgA antibodies detected in the samples showed cross-binding ability to multiple HA of different subtypes, most of which had phylogenetically close relationship with the subtypes of the respective immunogens. HA-specific IgA antibodies were only detected prominently in the samples of mice immunized intranasally. No difference was found in the repertoires (i.e., coverage of HA subtypes to which antibodies reacted) of the cross-reactivity between IgG and IgA antibodies. On the other hand, neutralizing activity of the serum samples were observed against homologous but not heterologous subtypes.

Although heterosubtypic neutralizing activity was not detected in the serum samples of immunized mice, antibodies secreted onto the mucosal surface may have a potential to neutralize virus infectivity by some mechanisms (e.g., inhibition of viral assembly and/or particle formation) which cannot be evaluated by an ordinary neutralization test. HA-specific antibodies cross-reactive to multiple HA subtypes detected in the mucosal secretions, especially IgA, might be important for heterosubtypic protection against influenza A viruses.

Poster Core time II at Lecture room 2 (Day 2: 16:15-17:15 on September 17)

P-32

Cross-protective peptide vaccine against influenza A viruses developed in HLA-A*2402 human immunity model

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Background : The virus-specific cytotoxic T lymphocyte (CTL) induction is an important target for the development of a broadly protective human influenza vaccine, since most CTL epitopes are found on internal viral proteins and relatively conserved. In this study, the possibility of developing a strain/subtype-independent human influenza vaccine was explored by taking a bioinformatics approach to establish an immunogenic HLA-A24 restricted CTL epitope screening system in HLA-transgenic mice.

Results : HLA-A24 restricted CTL epitope peptides derived from internal proteins of the H5N1 highly pathogenic avian influenza A virus were predicted by CTL epitope peptide prediction programs. Of 35 predicted peptides, six peptides exhibited remarkable cytotoxic activity *in vivo*. More than half of the mice which were subcutaneously vaccinated with the three most immunogenic and highly conserved epitopes among three different influenza A virus subtypes (H1N1, H3N2 and H5N1) survived lethal influenza virus challenge during both effector and memory CTL phases. Furthermore, mice that were intranasally vaccinated with these peptides remained free of clinical signs after lethal virus challenge during the effector phase.

Conclusions : This CTL epitope peptide selection system can be used as an effective tool for the development of a cross-protective human influenza vaccine. Furthermore this vaccine strategy can be applicable to the development of all intracellular pathogens vaccines to induce epitope-specific CTL that effectively eliminate infected cells.

Abstract

Poster Core time I at Lecture room 2 (Day 1: 16:30-17:30 on September 16)

P-33

Pathological examination of lung tissues in influenza A virus-infected aged mice

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Background: It has been reported that the elderly persons are known to be at highly risk from influenza (*Vaccine* 23: S1, 2005), and a decline or functional change in their immune system with aging, including a reduction in ability of T-cells to proliferate and reduced B-cell involved antibody responses. (*Dev Comp Immunol* 31: 407, 2007). The current study was performed in order to examine the pathological change of lung tissues of the influenza A-infected aged mice.

Methods: Young (8 weeks-old) and aged (108 weeks-old) C57BL/6 mice were intranasally inoculated with influenza A virus [A/PR/8/34 (H1N1), 1,000 plaque-forming units/mouse] Clinical symptom was monitored. Pathological and immunohistochemical analysis of the lung tissues was evaluated by 6 and 9 days post-infection (dpi).

Results: The body weight decrease of virus-infected mice was well correlated with sickness of the mice. Decrease of the body weight of aged mice was delayed compared to that of young mice. Pathological examination revealed that there were more inflammatory areas in lungs of young mice compared with in lungs of aged mice. The number of the polymorphonuclear neutrophils (PMNs) in the lung tissues was significantly higher than that of the virus-inoculated aged mice at 6 dpi. In addition, TUNEL positive cells were recognized in the virus-inoculated young mice at 6 dpi; however, those were not detected in aged mice. The numbers of the T-cells and B-cells were not significantly different between young and aged mice.

Conclusion: The rapid body weight decrease in young mice seems to be related to acute lung injury induced by influenza virus infection, which triggers an early inflammatory response characterized by the high influx of inflammatory cells with interstitial and alveolar edema, causing subsequently immunopathological damage against self-tissue. On the other hand, aged mice showed a delayed loss of body weight with limited lung tissue damage and diminished recruitment of PMNs to the alveolar and peri-bronchial region at the early stage of the infection.

Poster Core time II at Lecture room 3 (Day 2: 16:15-17:15 on September 17)

P-34

Studies on the Synthesis and Expression of Viral Glycoproteins from Borna Disease Virus and Avian Bornavirus in Transfected cell lines

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Borna disease virus (BDV) causes neurologic disease manifested by behavioral abnormalities in several animal species, and evidence suggests that it may be a human pathogen. BDV glycoprotein (BDVG) is posttranslationally cleaved by the cellular protease furin to generate two subunits of the N-terminal GP1 and C-terminal GP2. GP1 is involved in virus interaction with cell surface receptor and GP2 mediates a pH-dependent fusion event between viral and endosome membrane. BDVG expression is extremely low in BDV persistently infected cells and the cells transfected with eukaryotic expression plasmids, and these findings led to the view that BDV disseminates through cell-to-cell spread rather than virion release, although the details remain unclear. Recently, avian bornaviruse (ABV) was identified in parrots with proventricular dilatation disease. However, no information is available yet about the glycoprotein of ABV (ABVG). To investigate the role of these glycoproteins in propagation and pathogenesis of bornavirus infections, we characterized the synthesis and the expression of ABVG in the transfected cells and compared them with those of BDVG.

Characterization of ABVG: When the ABVG cDNA C-terminally tagged with FLAG epitope was transfected into 293T cells, the expression of two polypeptides with molecular masses of 92 and 42 kDa was observed. The 92-kDa and 42-kDa bands corresponded to the full-length ABVG (GPC) and its C-terminal cleavage product GP2, respectively. Deglycosylation studies demonstrated that both products predominantly contained endoglycosidase H-sensitive *N*-linked oligosaccharides. Furthermore, cell surface biotinylation assay showed that GP2 was expressed at the cell surface, whereas the vast majority of GPC did not reach the plasma membrane and was accumulated in intracellular compartments. Immunofluorescence microscopy showed that the FLAG signals were accumulated in the plasma membrane and the endoplasmic reticulum (ER). Morerover, ABVG-transfected cells formed syncytia when exposed to low pH. These results indicate that the ABVG GP2 is expressed at the cell surface so as to cause the cell-cell fusion, and suggest that the GP2 is responsible for the fusion event of virus particles with endosomal membranes.

Differential expression of BDVG and ABVG: We generated a series of chimeric proteins of BDVG and ABVG. Expression analysis demonstrated that substitution of the transmembrane segment (TM) of ABVG by that from BDVG remarkably reduced expression of the chimera, while expression of the chimeric BDVG possessing TM of ABVG was significantly increased. These findings suggest that the structure of the TM of BDVG affects plasma membrane expression of BDVG. On the other hand, northern blotting analysis exhibited that the expression level of the BDVG RNA was remarkably lower than that of the ABVG RNA in transfected cells, suggesting the presence of some mechanisms to restrain transcription of the BDVG gene. One of the possible mechanisms involved undesirable splicing using the donor-acceptor motif within the BDVG gene. To address this question, the studies are in progress in our laboratory.

Abstract

Poster Core time I at Lecture room 3 (Day 1: 16:30-17:30 on September 16)

P-35

Detection of Polyomaviruses from Vervet monkey and Baboon in Zambia

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Members of the family *Polyomaviridae* are non-enveloped icosahedral viruses with a circular double-stranded DNA genome approximately 5,000 bp in size. The life cycle of polyomaviruses (PyVs) still remains unclear and it is important to perform surveillance of known and unknown PyVs in wildlife. In this study, using DNAs extracted from vervet monkeys and baboons captured in Zambia, we attempted to detect PyVs.

DNAs were isolated from kidneys and spleens of vervet monkeys (n=50) and baboons (n=50) in Zambia, and subjected to a nested broad-spectrum PCR assay with primers for capsid protein VP1. A BLAST search showed that the PCR products approximately 250 bp to be over 80% homologous with those of VP1 in different PyVs at the nucleotide level of VP1 region. Thereafter, an inverse PCR using the tail-to-tail primer sets within the amplified VP1 fragments, was performed to identify whole PyV genomes. We successfully detected PyV genomes from vervet monkey (n=3) and baboon (n=2) out of fifty. Compared their genomes organization with other PyVs, all the genomes code viral regulatory proteins, including small and large tumor antigens, viral structural proteins, VP1, VP2, and VP3. A phylogenetic analysis using the PyV proteins by the neighbor-joining method revealed that most of these viruses were closely related to B-lymphotropic PyV (87-94%) or Simian agent 12 (88-94%). But only one virus which was detected from vervet monkey spleen was related to Chimpanzee PyV and it had 74% homology with that virus.

We detected PyVs from 3 vervet monkeys (spleen: 3, kidney: 1) and 2 baboons (spleen: 2, kidney: 1) out of fifty. Because of the virus which was detected from verevt monkey spleen had low homology (74%) with Chimpanzee PyV, it has a potential that this virus is a novel PyV. And also, according to the alignment results between this virus and known PyVs, we confirmed that the virus has extra 150 amino acids in the C-terminal tail of VP1 compared with other known PyVs except for Chimpanzee PyV.

Poster Core time II at Lecture room 3 (Day 2: 16:15-17:15 on September 17)

P-36

Possible origin of CSF antibodies induced by intrathecal immunization and apply to rabies control in experimental animals

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[Introduction]

Intrathecal (IT) immunization involves injecting antigens directly into the intraventricular, subarachnoid spaces or brain, to induce antigen-specific antibodies (Ab) in the cerebrospinal fluid (CSF). The objective of the present study is to investigate the origins of CSF Ab after IT immunization using experimental animals.

[Materials&Methods]

Adult, female, NZW rabbits were IT immunized with inactivated rabies virus. Serum Ab, cytokine expression, histopathology and immunohistochemistry of brains were analyzed and compared with control. Adult, female, ICR mice were challenged rabies virus (CVS strain) directly into the brain to estimate prophylactic effect of IT immunization within central nervous system (CNS).

[Results]

CSF Ab was rapidly induced after second IT immunization and TNF-alpha expression was also increased. Mononuclear cells including Ab-producing cells infiltrated multi-focally around the blood vessels of the brain and leptomeninges. Furthermore, subcutaneous (SC) immunization prior to IT immunization induced rapid and magnified Ab responses in the CSF compared with IT immunization alone. These results were confirmed by the fact that mice immunized SC prior to IT were resistant to intracerebral virus challenge.

[Conclusion]

The possible origin of CSF Ab is speculated both influx from serum and local production within CNS. Further, combined SC and IT immunization might be a more effective vaccination protocol for prophylaxis and treatment of rabies.

Abstract

Poster Core time I at Lecture room 3 (Day 1: 16:30-17:30 on September 16)

P-37

Chemokine improved anti-neurotropic virus immune response by attracting antibody secreting cells to the CNS

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【Objectives】
In our previous study, antibody secreting cells (ASCs) recruited to the brain following upregulated chemokines after IC immunization contributed to the suppression of neurotropic virus. In this study, we tried intracerebral chemokine injection to attract ASCs to the brain for control of neurotropic virus.

【Materials and methods】
Inactivated pseudorabies virus (PRV) or rabies virus (RV) was used for immunization. After secondary immunization, lymphocytes were collected from spleen and bone marrow (BM) to investigate kinetics of Ag-specific ASCs, and *in vitro* chemotaxis assay was performed with splenocytes. Intracerebral virus challenge after chemokine injection to the brain was done.

【Results】
The PRV-specific ASCs in BM increased gradually, though they were less than in spleen, and exceeded that in spleen at 21-25 days post secondary immunization (dpi). At 24 dpi, CXCL12 and cocktail chemokine (mixture of CXCL9, 10, 12 and 13) attracted PRV-specific ASCs most strongly, compared to CXCL9, 10 and 13 in *in vitro* chemotaxis assay and *in vivo* intracerebral chemokines injection experiment. Intracerebral injection of CXCL12 did not affect the survival rate in live PRV challenge, though it promoted long-term survival. But in live RV challenge CXCL12 increased the survival rate. On the other hand, intracerebral injection of cocktail chemokine increased the survival rate in both PRV and RV challenge.

【Conclusions】
Chemokine injection to the brain led to the approach of ASCs to the brain and contributed to the suppression of neurotropic viruses (PRV and RV).

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P-38

Characterization of protooncogene TCL1b as an Akt kinase co-activator

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The intracellular Akt signaling pathway is activated by various cytokines or growth factors and mediates intracellular signals to regulate a wide variety of cellular responses, including anti-apoptosis, proliferation, cell cycling, protein synthesis, glucose metabolism, and telomere activity. Genomic mutations, alterations of the Akt regulatory signaling pathway underlie various human diseases such as cancers, glucose intolerance (or diabetes mellitus), schizophrenia, and/or autoimmune diseases.

Recently, in addition to direct involvement in tumorigenesis by genetic alterations of human cancers, the Akt signaling network also underlies the clinical manifestation of various stages of tumorigenic viral infection, such as latent infection, chronic infection, and malignant transformation of the Epstein-Barr virus, the Hepatitis C virus, the Hepatitis B virus, or the Human Immunodeficiency Virus (HIV).

We have previously demonstrated that protooncogene TCL1 (T cell leukemia 1), translocated in human T cell prolymphocytic leukemia (T-PLL), is an Akt kinase co-activator. Three TCL1 isoforms have been identified, namely TCL1, TCL1b, and MTCP1 with predicted molecular weight of 13-15 kDa. The TCL1 families share a relatively high degree of amino acid homology (30-50%) and a unique, symmetrical β-barrel structure. In human T-PLL, both TCL1 and TCL1b genes are activated by juxtaposition onto the T-cell receptor α or β loci. Therefore, it remains unclear whether TCL1b itself bears oncogenicity. In this study, we investigated whether and how TCL1b might have similar function as Akt kinase co-activator. In co-immunoprecipitation assays, TCL1b co-immunoprecipitated with Akt. In vitro Akt kinase assays, TCL1b enhanced Akt kinase activity. Moreover, by Agilent Expression Array analysis, both TCL1 and TCL1b showed highly significant homologous gene induction pattern as myr-Akt, a constitutive active form of Akt. The observations are consistent in Gene Ontology, KEGG pathway, and cluster analysis. These observations together suggested that analogous to TCL1, TCL1b also functions as an Akt kinase co-activator, and possibly plays an active role in oncogenicity in human T-PLL.

Furthermore, anti-viral activity by suppression of Akt activity may provide alternative therapeutic opportunities that can prevent malignant transformation by suppression of tumorigenic viral infection rather than directly targeting anticancer activities via oncogene.

Abstract

Poster Core time I at Lecture room 3 (Day 1: 16:30-17:30 on September 16)

P-39

Molecular characterization of immunoinhibitory factors PD-1/PD-L1

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An immunoinhibitory receptor, programmed dath-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 and tumors. The blockade of the PD-1/PD-L1 pathway by antibodies specific to either PD-1 or PD-L1 resulted in the reactivation of immune reactions, and is expected to be applied to new therapies for the chronic infectious diseases and tumors. However, few functional analyses of these molecules have been reported for domestic animals.

In the previous study, we cloned bovine PD-1 and PD-L1, and performed PD-L1 expression analysis in bovine leukemia virus (BLV)-infected cattle. The proportion of PD-L1 positive cells in Peripheral blood mononuclear cells (PBMCs) correlated positively with prediction markers for the progression of the disease, whilst on the other hand, it inversely correlated with the degree of IFN-g expression. Blockade of the PD-L1 *in vitro* by PD-L1-specific antibody upregulated the production of IL-2 and IFN-g, and correspondingly, downregulated the BLV provirus load. These data suggest that PD-L1 induces immunoinhibition in disease progressed cattle during chronic BLV infection. However, it is not directly demonstrated that the PD-1/PD-L1 pathway induces immunoinhibition in BLV infected cattle. So, in this study, we generated PD-1 and PD-L1 expressing cells for detection of immune regulatory function of bovine PD-1/PD-L1 pathway. PBMCs isolated from BLV-infected cattle were cultivated with PD-1 or PD-L1 expressing cells. Cytokines, such as *IL-2* and *IFN-g* mRNA were upregulated in PBMCs cultivated with PD-1 expressing cells, and downregulated in those with PD-L1 expressing cells. And the proliferation capacity was enhanced in PBMCs cultivated with PD-1 expressing cells, and inhibited in those with PD-L1 expressing cells. Therefore, PD-1/PD-L1 pathway would be a potential target for developing immunotherapies against BLV infection.

Poster Core time II at Lecture room 3 (Day 2: 16:15-17:15 on September 17)

P-40

The role of non-receptor tyrosine kinase TYK2 in Toll-like receptor signaling

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Cytokines play an important role in the communication between the innate and the adaptive immune systems in order to protect hosts from invading pathogens. The innate immune system uses pattern recognition receptor such as Toll-like receptor (TLR) and retinoic acid-inducible gene-I (RIG-I) to detect conserved microbial molecules, and then induces pro-inflammatory cytokine gene expression such as interleukin (IL)-1β, IL-6, IL-12 and tumor necrosis factor (TNF)-α. These cytokines induce T-cell differentiation and activation of adaptive immune responses. Activated adaptive immunity produces cytokines such as IL-2, interferon (IFN)-γ and IL-17, which induce further activation and mobilization of innate immune cells. In these cytokine responses, tyrosine kinase 2 (TYK2) plays an important role in various cytokine signaling. However, the contribution of TYK2 to cytokine production from innate immune cells is still unknown. In this study, we examined the requirement of TYK2 for TLR-induced pro-inflammatory cytokine productions and intracellular signaling mediated by nuclear facter (NF)-κB and mitogen-activated protein (MAP) kinase which lead to cytokine gene expressions in innate immune cells.

To investigate TLR-induced pro-inflammatory cytokine production, we used heat-killed *Propionibacterium acnes* (*P. acnes*) as a TLR ligand. In *Tyk2* knock-out (KO) mice, *P. acnes*-induced granuloma formation and IFN-γ production which enhances granuloma formation were impaired. It is known that induction of IFN-γ production is enhanced by IL-12, which is produced mainly by antigen presenting cells such as macrophages (Mϕs) and dendritic cells (DCs). We found that *P. acnes*-induced expression of IL-12 mRNA was also diminished in *Tyk2* KO Mϕs. Furthermore, *P. acnes*-induced activations of NF-κB and MAP kinase in *Tyk2* KO Mϕs were diminished. In TLR signaling, phosphoinositide 3-kinase (PI3K)-AKT pathway negatively regulates the activation of NF-κB and MAP kinase via Src family kinase LYN. Interestingly, TYK2 was associated with LYN in 293T cells and activation of AKT and LYN were decreased in *Tyk2* KO Mϕs. Taken together, these results indicate that TYK2 enhances the activation of NF-κB and MAP kinase by suppressing the LYN-mediated activation of PI3K-AKT pathway and consequently positively regulates *P. acnes*-induced IL-12 production in Mϕs.

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