Activities of Mahidol Osaka Center for Infectious Diseases
~Dengue, Chikungunya and other viruses~

Tamaki Okabayashi, DVM, PhD
Activities of
**Mahidol Osaka Center for Infectious Diseases**
~Dengue, Chikungunya and other viruses~

- What is MOCID?
- Basic concepts
- Research projects
  - Collaboration researches
  - MOCID researches
Thailand-Japan Collaboration Projects

~ Mahidol University --- Osaka University ~

Faculty of Tropical Medicine

BIKEN Endowed Dept. of Dengue Vaccine Development

Research Institute of Microbial Diseases

MOCID

J-GRID

JST/JICA SATREPS

JST/JICA
J-GRID
Japan Initiative for Global Research Network on Infectious Diseases

Vietnam
- Vietnam-Nagasaki University Research Center
  National Institute of Hygiene and Epidemiology (NIHE)
- Vietnam-International Medical Center of Japan Research Center
  Bach Mai Hospital and other hospitals

Ghana
- Ghana-Tokyo Medical and Dental University Research Center
- Noguchi Memorial Institute for Medical Research, University of Ghana

India
- India-Okayama University Research Center
  National Institute of Cholera and Enteric Diseases (NICED)

Thailand
- Thailand-National Institute of Animal Health Research Center
  National Institute of Animal Health (NIAH)

China
- China-The University of Tokyo Research Center
  Institute of Biophysics, Chinese Academy of Sciences (IBPCAS)
  Institute of Microbiology, Chinese Academy of Sciences (IMCAS)
  Harbin Veterinary Research Institute (HVR), Chinese Academy of Agricultural Sciences (CAAS)

Zambia
- Zambia-Hokkaido University Research Center
  Samora Machel School of Veterinary Medicine, the University of Zambia

Indonesia
- Indonesia-Kobe University Research Center
  Tropical Disease Center (TDC), Airlangga University

Philippines
- Philippines-Tohoku University Research Center
  The Research Institute for Tropical Medicine (RITM)
MOCID committee

- Chairwoman: FTM Dear Dr. Yaowalark Sukthana
- Thai-side: Dr. Pongrama Ramasoota, CERA
- Dr. Emsri Pongponratn, Deputy Dean for International Cooperation and Networking
- Japanese-side: Dr. Konishi, BIKEN unit
- Dr. Okabayashi, MOCID unit
Basic Concept of MOCID unit

Surveillance of Viral Diseases

Basic Research on immunopathology ~Virus and Host~

Monoclonal Antibody

Therapeutic

Diagnostics

Evaluation in model system

Evaluation using clinical samples
Basic Concept of MOCID

TARGETS
- DENV & CHIKV
- Other virus
- Skin
- Langerhans cell
- Keratinocyte
- Monocyte
- Macrophage
- Endothelial cell
- Joint Pain

QUESTIONS
- Epidemiology
  - Clinical strain (Sever/Mild)?
  - Lab. strain?
  - Mosquito strain?
- Effects on viral propagation or cytokine production?
  - Mosquito’s salivary gland protein?
- Function of Antibody?
  - ADE
  - Autoimmune antibody
- Antibody
  - Vaccine
  - Diagnostic kit
- Pathogenic factors?
  - Virus
  - Soluble factor
  - Apoptosis etc
Facility of MOCID

50th Anniversary
Chalerm Phrakiat Building
12th Floor
- What is MOCID?
- Basic concepts
- Research projects
  - Collaboration researches
  - MOCID researches
Collaboration Research titles with MU

Dengue virus monoclonal antibody

Human MAb to neutralize all DENV serotypes using lymphocytes from patients at acute phase of the secondary infection. Settgapramote et al., BBRC, 2012

Dengue virus neutralization and antibody-dependent enhancement activities of human monoclonal antibodies derived from dengue patients at acute phase of secondary infection. Sadaki et al., Antiviral Res. 98, 423-31.

Cross-reactivity of human monoclonal antibodies generated with peripheral blood lymphocytes from dengue patients with Japanese encephalitis virus. Pipattanaboon C et al., Biologics 7; 175-87.

Characterization of **the salivary gland proteins** of the Aedes aegypti, Ae. albopictus and Ae. scutellaris. Morales Vargas RE et al. JITMM, 2013 *Consolation Prize*

Detection of **Enteric Virus** in flood water from the 2011 Thai Flood. Ngaosuwankul N et al., Jpn, J. Infect Dis. 2013

Detection of **hepatitis E virus** in deer and monkey in Thailand Ngaosuwankul N et al., JITMM, 2013
Hospital of Tropical Diseases
Dengue research group
Molecular diagnosis of DENV and CHIKV
Dr. Udomsak Silachamroon, Dr. Watcharapong Piyaphanee…..

Dept. Tropical Pediatrics
Dr. Arunee Sabchareon

Vaccine development
The comparative analysis of CYD tetravalent DNEV vaccine and Thai-DENV-2
Kuwahara et al., JITMM 2013,

Pathogenisty of DNEV
Antibody dynamics in dengue patients
→Relationship between ADE and DENV severity

Training scheme at MOCID
Dr. Khuanchai Koompapong, Dr. Supawat Chatchen,
Ms. Jittraporn Pathanamahapoom
Faculty of Science, Dept. Pharmacology

Pathogenicity of Flavivirus

Flavivirus Induced Reactive Oxygen Species (ROS) in Rat Microglia Cells
Dr. Noppawan Phumala Morales, Ms. Samaphorn Maneethep

Center for Vaccine Development

Evaluation of CHIKV IC diagnostic kit
Dr. Sutee Yoksan
Collaboration research titles with Oversea countries

**Indonesia, Japan**

**Indonesia - Japan - Thailand collaboration research**

Airlangga Univ.  Kobe Univ.  NIH  Osaka Univ.  FTM MU.

**MOCID**

Generation of human monoclonal antibody against **DENV/CHIKV**
Development of IC diagnostic kits for **DENV/CHIKV**

**Japan**

**Primate Research Institute**  
**Kyoto University**

Collaboration research

**Faculty of Veterinary Science,**  
**Rakuno Gakuen University, Hokkaido**

Collaboration research / Academic exchange

**Evolution of Primate and Microbiology**

**Surveillance of HEV**
Committee of the Thesis Proposal Examination
Mahidol University

Epitope mapping of human monoclonal antibodies against Dengue virus
Ms. Chonlatip Pipattanaboon, CEAR, FTM

Seroprevalence of antibodies against Japanese encephalitis virus in wild and domestic animals of Thailand
Ms. Mayurin Laorujisawat, Dept. Microbiology, Faculty of Public Health

Effect of iron chelators on intracellular ROS production in iron overloaded microglia cells
Ms. Samaporn Maneethep, Dept. Pharmacology, Faculty of Science

Expression and characterization of antimicrobial peptide gambicin from Culex quinquefasciatus in Pichia pastoris
Ms. Phanthila Sirichaiyakul, Dept. Tropical Nutrition and Food, FTM

Flavivirus Induced Reactive Oxygen Species (ROS) in Rat Microglia Cells.
Mr. Lattapon Suwanprinya Dept. Pharmacology, Faculty of Science

Visiting associate professor, Dept. Medical Entomology, FTM, Nov, 2013
Research titles of MOCID

Dr. Yoshiharu Matsuura
Dr. Tamaki Okabayashi

Dr. Mikiko Sasayama
Dr. Miwa Kuwahara

Dr. Orapim Puiprom
Ms. Panjaporn Chaichana

Ms. Nantarat Chantawat
(Researcher assistance)

Ms. Thanita Ritpinyo
(secretary)
Research titles of MOCID

Publication list

**Epidemiology**

Co-existence of major and minor viral populations from two different origins in patients secondary infected with DENV2 in Bangkok. Puiprom et al., BBRC, 2011

**Mosquito**

Characterization of CHIKV infection of a human keratinocyte cell line: Role of mosquito salivary gland protein in suppressing the host immune response.
Puiprom et al., IGE, 2013

**Monoclonal antibody analysis  → diagnostics, therapeutic, pathogenicity**

Human MAb to neutralize all DENV serotypes using lymphocytes from patients at acute phase of the secondary infection.
Settgaramote et al., BBRC, 2012

Limited cross-reactivity of mouse MAbs against DENV capsid protein among four serotypes.
Noda et al., Biologics, 2012
Research titles of MOCID

In preparation

Low levels of antibody-dependent enhancement in vitro using viruses and plasma from dengue patients
Panjaporn et al., PLOS ONE, under review

Chikungunya Virus was isolated in Thailand even after the 2009 outbreaks
Sadayama et al., EID, under review

Comparative analysis of Dengue virus serotype 2 and CYD tetravalent dengue vaccine strains in Thailand
Kuwahara et al., Vaccine, under review

Neutralization of chikungunya virus by a monoclonal antibody recognizing envelope 1 protein that inhibits the virus release process.
Promsin et al., in preparation

Development of Rapid immunochromatography test kit to detect the Chikungunya
Okabayashi et al., in preparation
Conclusion

❖ What is MOCID? 

❖ Basic concepts

❖ Research projects

Collaboration researches

Basic & Clinical research groups
  Mahidol University (FTM, FSci, FVS ……)
  Indonesia (Airlangga-U)
  Japan (Osaka-U, Kyoto-U, Rakuno-U)

MOCID researches

DENV, CHIKV, Enteric Virus, Zoonotic Virus

MAbs research → Rapid diagnostic kit
Epidemiology
Pathogenesis
MOCID

DOORs are OPEN!

Any ideas and interesting researches will pop up in your minds, our Lab are appreciating you to step on our doors and you will be more welcome.
ขอบคุณคุณ

Thank you for your attention

Opening Ceremony, July, 2011
Development of immunochromatography diagnostic kits for CHIKV

Mouse MAb against CHIKV

Monoclonal Antibody

Sensitivity/Specificity evaluation *in vitro*

DENV 1~4 (Lab strain, Clinical strain)
CHIKV
Other virus (Flavivirus, Alphavirus etc….)

Clinical evaluation

Patient samples (Thailand, Indonesia)
CK47 MAb blocks at releasing step of CHIKV, but not entry and replication steps.

**CK47 MAb-escape mutant of CHIKV**

Incubation with/without CK47 MAb for 8th round

Wild type (x8)  Escape mutant (x8)

**Env. 1**

<table>
<thead>
<tr>
<th>WT (x8)</th>
<th>GNMPISIDIPGAAATRVDAPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>EM (x8)</td>
<td>GNMPISIDIPGAAATRVDAPS</td>
</tr>
</tbody>
</table>

CHIKV CP10 EM: E286G
CK47 MAb is a post-entry neutralization of CHIKV infection
→ Development of therapeutic monoclonal antibodies
→ Useful to clarify the mechanism of CHIKV releasing
ADE and Quasispecies in DENV infection

Ms. Panjaporn Chaichana
CEAR, JICA

DENV-HuMAb production
Settgapramote et al., 2012
Sasaki et al., 2012

DENV quasispecies
Puiprom et al., 2011

Characterization of MAb
Epitope
Neutralize level
Cross-reactivity
ADE

Which combination relate with Neutralization and Enhancement?
Post-entry neutralization of CHIKV by Mo-MAb

Dr. Orapim Puiprom, Dr. Miwa Kuwahara

CHIKV: Specific treatment / vaccine are not available.

- CHIKV infection is thought to confer lifelong immunity.
- Convalescent serum from CHIKV patient protected mice from lethal infection

Neutralizing activity of CHIKV-specific mouse MAbs

CK47 MAb inhibits virus spreading, but not entry?

Effect of CK47 MAb treatment on plaque formation of CHIKV

1. Virus-Ab complex and post entry
2. Pre-entry
3. Entry process
4. Post-entry
Characterization of CHIKV infection of keratinocyte
Role of mosquito salivary gland protein

Dr. Orapim Puiprom, MOCID
Dr. Ronald E. Morales, Dept. Medical Entomology

CHIKV in human skin cells?
CHIKV; isolated in Thailand, 2010
HaCaT cell lines; human keratinocyte cell line

The effects of SGP on
CHIKV replication?
Immune regulation?

uninfected CHIKV (24 h m. o. i. 1)
CHIKV works together with SGP to ensure the virus replicates in skin cells and escapes the host immune system by suppression of IL-8 production.
Detection of enteric virus in flood water of 2011-Thai-Flood

Prevalence of enteric viral genes in flood water samples collected in Thailand

<table>
<thead>
<tr>
<th>Area (tested no.)</th>
<th>HAV</th>
<th>HEV</th>
<th>Enterovirus</th>
<th>Rotavirus</th>
<th>Norovirus</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salaya (70)</td>
<td>5* (7.1)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>8* (11.4)</td>
<td>12 (17.1)</td>
<td>25 (35.7)</td>
</tr>
<tr>
<td>Others (30)</td>
<td>2 (6.6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (3.3)</td>
<td>2 (6.6)</td>
<td>5 (16.6)</td>
</tr>
<tr>
<td>Total (100)</td>
<td>7 (7)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>9 (9)</td>
<td>14 (14)</td>
<td>30 (30)</td>
</tr>
</tbody>
</table>

The First report in Thailand
Presence of enteric virus gene in flood water
Detection NV gene from environmental water

Pornsawan et al., Intervirology. Under review.
Patients

Blood (10 ml) Cell fusion

Hybridoma clones producing HuMAbs

PBMCs with fusion partner cells (SPYMEG)

Y Y Y Y Y
Development of immunochromatography diagnostic kits for mosquito-borne infections with DENV and CHIKV

Human MAb against DENV

Mouse MAb against CHIKV

Monoclonal Antibody

Future Task

Sensitivity/Specificity evaluation in vitro

Clinical evaluation

DENV 1~4 (Lab strain, Clinical strain)
CHIKV
Other virus (Flavivirus, Alphavirus etc....)

Patient samples (Thailand, Indonesia)
Collaboration research titles with Oversea countries

Indonesia, Japan

Indonesia - Japan - Thailand collaboration research

Airlangga Univ.  Kobe Univ.  NIH
Osaka Univ.  FTM MU.

MOCID
Generation of human monoclonal antibody against DENV/CHIKV
Development of IC diagnostic kits for DENV/CHIKV

Japan

Primate Research Institute
Kyoto University

Collaboration research

Faculty of Veterinary Science,
Rakuno Gakuen University, Hokkaido

Collaboration research /Academic exchange

Evolution of Primate and Microbiology

Surveillance of HEV
Research groups with Mahidol University

Dengue Research Group

Dr. Pratap Singhasivanon, Dr. Pongrama Ramasoota, Dr. Udomsak Silachamroon

**MOCID: Molecular diagnosis of DENV/ CHIKV infection**

Collaboration research group on Zoonotic diseases in Thailand

FTM, Faculty of Veterinary Science, Zoonotic Diseases Control Center, MOCID

**MOCID: CHIKV, HEV**

Enteric group

Dr. Yaowalark Sukthana, Dr. Chalit Komalamisra

**MOCID: Viral Enteric Diseases (HEV, HAV, Norovirus etc...)**