ADC Letter
for Infectious Disease Control

Online ISSN 2424-0907
Print ISSN 2189-5171

Vol.4 No.1 2017.1.1

Asia International Institute of Infectious Disease Control,
Teikyo University
〒173-8605 東京都板橋区加賀2-11-1
Tel：+81-3-3964-1211 Fax：+81-3-3964-2580
E-mail：office-adc@med.teikyo-u.ac.jp
http://www.teikyo-u.ac.jp/affiliate/laboratory/adc_labo/

TEIKYO

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Medal for People's Health in Vietnam

EDITORIAL

Report to officers at JICA-Vietnam with Drs.Duong and Thuy

鈴木和男所長と河内正治副所長（帝京大学 アジア国際感染症制御研究所）は、2016年11月3日付でベトナム保健省からMedal for People's Healthを授与されました。授賞式は、同11月4日ハノイMedia Hotelにて行われました。これは、2005年のH5N1鳥インフルエンザの感染症調査から11年間にわたり厚生労働省：厚生労働科学研究費、文部科学省・日本学術振興会（JSPS）、科学技術振興機構（JST）・AMED：e-ASIA「インフルエンザおよび肺感染症研究での国際研究の支援」により進めてきたことが評価され、ベトナム国民の健康に役立ったことかと思います。本受賞に際し、Liem前院長、Hai現院長、Dung前副院長、Dien現副院長およびThuy研究部長に感謝します。また、本号にも掲載しましたが、帝京大学とベトナム国立小児病院およびハノイ医科大学との単位互換を実践することもできました。今後も、ベトナムをはじめとするアジア諸国の医療機関と帝京大学との連携をますます進めて行けることを願っています。

Director Kazuo Suzuki and Vice-Director Shoji Kawachi in Asia International Institute of Infectious Disease Control (ADC), Teikyo University have been awarded the Medal for People's Health from the Ministry of Health, Vietnam on November 3rd, 2016. The ceremony was held on the next day in Media Hotel in Hanoi. The medals were given for acknowledgements for joint researches in the National Children's Hospital in Hanoi (NCH). The collaborating studies have been conducted by the Ministry of Health, Labour and Welfare Grant, Ministry of Education, Culture, Sports, Science and Technology, JSPS, and JST-AMED project "International studies on Influenza and pulmonary diseases" for 11 years since Research for the H5N1 avian influenza research program in 2005. It is very honored that they contributed to Vietnamese health. We appreciate Drs. Liem, Hai, Dung, Dien and Thuy in the NCH for their contribution. In addition, we developed tight connections under the Agreement for Academic Exchange and Cooperation between Teikyo University and NCH, and Teikyo University and Hanoi Medical University in Vietnam in June-July 2016. We are hoping to continue collaborations with researchers in the medical and science fields in Vietnam and other Asian countries.

ＡＤＣ研：鈴木和男所長と河内正治副所長がベトナム保健省より「Medal For People's Health」を受賞

Prof. Suzuki and Prof. Kawachi were awarded the Medal For People's Health in Vietnam, Nov. 3, 2016

帝京大学アジア国際感染症制御研究所、鈴木和男所長・河内正治副所長が、2016年11月3日「Medal For People's Health」を受賞した。これは、ベトナムの医療発展に多大な貢献をした専門家に贈られる栄誉ある賞だ。平成18年から始まった高病原性鳥インフルエンザとその死因となる（ARDS）に関する研究をベトナム国立小児病院と連携して行い、10年以上にわたる研究開発活動がベトナム政府から高く評価された。

鈴木和男所長は、ベトナム国立小児病院病理部に保管してあった病理標本ブロックを使い、インフルエンザの重症化の病態解明を進め、さらにそれにかかわるサイトカイン・ケモカインの微量免疫学的測定法を検査室に導入、病院の免疫学・生化学及び分子生物学的検査の高度技術化を推進した。その後、ベトナム国立小児病院Thuy Phung研究員を千葉大学大学院医学研究院に大学院生として迎え、指導した。「病態にかかわるサイトカインの役割」を明らかにし、博士号を取得させた。Thuy博士は、現在鈴木所長がAMEDのリーダーをつとめているe-ASIAプロジェクトのベトナム側プロジェクトリーダーとして活躍している。

河内正治副所長は、高病原性インフルエンザの臨床的特徴を明らかにし、その主な死因が重症ARDSであることを明らかした。また、大量γグロブリン療法、体外式膜型人工肺（ECMO）などの新しい治療法をいち早く導入し、ベトナム国立小児病院の治療技術向上を支援してきた。

現在も引き続き連携研究を行っており、今後もさらなる成果が得られることが期待される。

受賞者を代表して感謝のスピーチ（鈴木和男）

河内正治（左）鈴木和男（右）

受賞コメント

鈴木和男

「2005年H5N1鳥インフルエンザの調査以後、11年間にわたり国立小児病院と厚生労働省：厚生労働科学研究費、文部科学省・日本学術振興会（JSPS)、科学技術振興機構（JST）・AMED：e-ASIA=インフルエンザおよび肺感染症研究での国際研究の支援により進めてきたことで評価され、ベトナム国民の健康に役立ったことをうれしく思います。同時に、Liem前院長、Hai現院長、Dien現副院長およびThuy研究部長に感謝します。今後もベトナムから帝京大学大学院医学研究科に国費留学生として迎えている大学院生の博士号取得に尽力するとともに、ベトナム国民の健康に寄与したいと考えております。」

河内正治

「高病原性鳥インフルエンザの研究班は、2006年から今年で11年目になります。この8年間あまり研究班の主任を任せて頂きました。このベトナム国立小児病院との共同研究がベトナム政府にも評価されて、歴代班長に対してこのような名誉ある賞をいただけたのだと思います。継続は力なりです。今後もさらなる連携研究に携わっていきたいと思います。日本国政府の研究援助に感謝致します。」

帝京大学大学院公衆衛生学研究科の紹介

帝京大学大学院公衆衛生学研究科
帝京大学アジア国際感染症制御研究所（ADC） 井上まり子

公衆衛生は「公衆ノ生ヲ衛（まも）ル」と書き、人々の集団をいかに健康にするのかを実践する学問領域です。感染症制御に関しては古い歴史があり、その予防から疫学調査、調査システムづくり、法律や政策づくりなど様々なところで活かされています。

帝京大学では2011年に公衆衛生の専門職大学院であるSchool of Public Health（SPH）を開設しました。日本では第4校目（私立大学では初）の大学院であり、設立当初から世界のSPH教育基準である疫学、生物統計学、行動社会学、環境産業衛生学、保健政策管理学の5つの基本領域を体系的に学ぶ専門職大学院として教育を行ってきました。国内外で通用するMaster of Public Health（MPH）とDoctor of Public Health（DrPH）の人材育成に励んでいます。特に教育では、論文を執筆することだけではなく、得られた疫学調査などによるデータ分析結果をもとに解決策を立案して実践していくという問題解決型アプローチを重視しています。また、公衆衛生の実務家を育てることから、マネジメント能力、リーダーシップ、アドボカシーなどからなるコンピテンシーを伸ばす教育を実践しています。

科学的根拠をもとにいかにして社会をよくするかは、私たち大学に求められる重要な役割です。教員も学生も特に上記5領域に専門を持って研究を行っております。私自身は特に社会疫学の研究を中心にかかわっており、社会経済的要因（特に非正規雇用などの労働）が健康に及ぼす影響について国内外のフィールドやデータに基づき研究しています。これからもグローバルにそして日本の地域でも活躍できる人材育成に努め、研究が社会に活かされるよう活動していく所存です。

Teikyo University Graduate School of Public Health
Mariko Inoue, MPH. PhD

Public Health is “the science and art of preventing disease, prolonging life and promoting health through organized efforts and informed choices of society, organizations, public and private, communities and individuals” (Winslow, 1920). It is the duty of this discipline to identify the causes of disease detrimental to public health, to plan interventions to remove the causes of illness, and to take actions to maintain public health. In terms of infectious disease control, public health has a long history of contributions to its prevention, epidemiological study, creation of survey systems, and establishment of laws and health-related policies.

Teikyo University founded a professional Graduate School of Public Health (SPH) in 2011. Our school is the fourth (the first among private universities) SPH in Japan. At the very beginning of its foundation, we set up global standard curricula to provide education from five core fields of public health; namely, Epidemiology, Biostatistics, Environmental and Occupational Health, Social and Behavioral Sciences, and Health Policy and Management. We now offer two professional degree courses; Master of Public Health (MPH) and Doctor of Public Health (DrPH).

In terms of education, we put emphasis on a problem-solving approach, which suggests and practices interventions based on scientific evidence taken from the epidemiological and health policy research. Moreover, a number of complex public health problems at present require professionals to possess additional skills and competency in areas such as management, leadership, advocacy, and so on. We also initiated competency-based education in SPH with the aim of promoting public health professional school educational reform.

How we use scientific evidence to create a better society and healthier people is an indispensable role of universities. All the faculty members and graduate students undertake research on topics related to the five core fields described above. I myself mainly participate in research on social epidemiology, with a focus on how socioeconomic causes influence our health. My main research topic is how precarious work influences health. Through such research and education, we would like to further contribute to global and community health in the future.

Member of SPH

新任の挨拶

アジア国際感染症制御研究所 副所長　**河内正治**

2016年10月1日付でアジア国際感染症制御研究所副所長、医学部麻酔科学講座教授、附属病院安全管理部長として赴任いたしました。私は1980年広島大学医学部を卒業し、その後麻酔科医・救急医・集中治療医として広島大学医学部附属病院、広島県立広島病院、松江赤十字病院で臨床診療に携わって参りました。2002年に国立国際医療センターに赴任し、2004年より2014年は手術部長・集中治療部総合室長（管理部門長）として活動しました。2005年にベトナム国に出かけた際に鳥インフルエンザ患者に遭遇し、2006年の夏からADC研所長の鈴木和男先生（当時、国立感染症研究所）と一緒に鳥インフルエンザの引き起こす重症ARDSに関する研究を開始しました。2006年後期からは国立感染症研究所に客員研究員としてマウスの実験にも参加することが可能になり、感染症一般に対する関わりが非常に増えました。2007年から2014年まで厚生労働省の高病原性鳥インフルエンザによるARDS研究班班長として、ハノイ国立小児病院との共同研究を行いました。臨床医としても非常に有意義な研究でしたが、何よりも鈴木先生をはじめとする基礎の先生たちと一緒に行動し一緒に考える経験は、臨床医師としてのみならず医学者として大きな財産となり、大変に勉強をさせていただきました。今後の医学の発展には、この基礎と臨床が共同で行い一緒に考えること、が非常に大切であると痛感いたしました。

東南アジアでは、現在まで引き続いている鳥インフルエンザ研究において、さまざまな感染症に出会いました。やはり、日本はアジアのオピニオンリーダーとして、新興再興感染に立ち向かう必要があり、国際共同研究を推進するにあたってアジア国際感染症制御研究所の果たす役割は大きいと言えます。アジア国際感染症制御研究所での唯一の臨床医師所員として、日本の感染症対策に役立ちたいと考えています。

Inauguration Address

Shoji Kawachi
Vice Director of Asia International Institute of Infectious Disease Control, Teikyo University

As of October 1st 2016, I was appointed as vice director of Asia International Institute of Infectious Disease Control, professor of Department of Anesthesiology of School of Medicine and director of safety management of Teikyo University Hospital.

I graduated from school of medicine, Hiroshima University in 1980 and had worked for Hiroshima University Hospital as an anesthesiologist, an emergency physician and an intensive care physician. In National Center for Global Health and Medicine, I had been engaged as a surgery manager and a general manager of ICU from 2004 to 2014. In 2005, I encountered the patient with avian influenza in Vietnam and I started the study of severe ARDS introduced by avian influenza virus, with professor Kazuo Suzuki, director of Asia International Institute of Infectious Disease Control (at that time, he served at National Institute of Infectious Diseases). I gradually increased connection with general infectious diseases because I was a visiting scholar of National Institute of Infectious Diseases and I could join the mouse study there. I collaborated with the National Children's Hospital in Hanoi, Vietnam on the research of ARDS caused by highly pathogenic avian influenza virus from 2007 to 2014. I worked as a research leader in that project of Ministry of Health, Labour and Welfare, Japan. It was useful for my clinician career. What is more, the experience of studying and thinking together with other doctors and researchers including professor Kazuo Suzuki was precious asset for my work as a doctor not only a clinician. I can declare that it is quite important to collaborate together between basic research and clinical research for advances in medicine.

In Southeast Asia, I have studied various inflectional diseases through the ongoing avian influenza research. Japan should be an opinion leader and face up to emerging/re-emerging infectious diseases of common concern. Therefore Asia International Institute of Infectious Disease Control will play a big role in promotion of international collaborative research. As I am the only clinician in Asia International Institute of Infectious Disease Control, I will do my best to contribute to the infection control measures in Japan.

医学部５年生
公衆衛生学実習【19: ベトナムでの感染症】と連携
Training for Medical Students of Teikyo University

August 14th-20th, 2016

帝京大学医学部５年生のベトナム実習

東南アジア地域の感染症対策について、臨床・国際保健・予防医学、医療システムの観点から学習・研修しました。具体的には、ベトナム国立小児病院、国立ハノイ医科大学を訪問し、実習・研修およびグループディスカッションを通じて、①世界やアジアで発生している感染症の実情を理解し、②主要な感染症の予防・治療方法を学び、③我が国の感染症の世界での位置づけと防御について考えるとともに、④国際的視野に立った医療人としての役割を考えることについて実施しました。本実習・研修により、両機関から受講票が授与された。

Training of the Medical Students in Vietnam

Medical students in Teikyo University have learned the actual circumstances of infectious disease control in East-Asian countries from viewpoints of clinical medicine, international public health, preventive medicine and medical systems. They visited NCH and HMU in Vietnam, and then understood: 1) patients with infectious diseases, 2) prevention of the major infectious diseases, 3) facts of control procedures in infectious diseases for Japanese patients. Finally, 4) they will become medical staff in international perspective through training and discussion with local staff. After the training in Vietnam, the certificates from both institutions were given to each student.

Exchange program between Teikyo University and NCH and HMU

National Children's Hospital (NCH)

Hanoi Medical University (HMU)

目　的

- 世界やアジアで発生している感染症の実状を視察し、今後の医療活動に役立てる。
- 国際的視野にたった医療人をめざす。

実習概要

臨床実習、国際保健・予防医学、医療システム・アクセスの観点も含め学習

実習・研修する大学、病院、関連施設:

- National Children's Hospital（NCH、旧NHP:国立小児病院）
  ICU、呼吸器、循環器、感染症、救急、臨床疫学、他
- 国立ハノイ医科大学:Lectures:感染症、感染症疫学
- 日本大使館（在ハノイ）、JICA Vietnam

Coordinator：
鈴木和男（アジア国際感染症制御研究所　所長), Kazuo Suzuki
豊田彰史（医学部小児科　講師), Akifumi Toyoda
河内正治（ADC客員教授（現副所長)), Shoji Kawachi
Cooperation：関　順彦　（腫瘍内科病院教授), Nobuhiko Seki

Local Staff in Hanoi：
NCH：Hai病院長, Dien副院長,
Dung部長, Phuc部長,
Thuyラボチーフ, 各部長
HMU：Van副学長, 他

実習スケジュール

Schedule for Medical Students

Group 1	Mon	Tue	Wed	Thu	Fri
AM	Meeting	Respiratory	ER	ICU	Closing
PM	Laboratory	Cardiovascular	NICU	ID	Lecture at HMU
Group 2	**Mon**	**Tue**	**Wed**	**Thu**	**Fri**
AM	Meeting	ICU	NICU	ER	Closing
PM	Laboratory	ID	Cardiovascular	Respiratory	Lecture at HMU

Responsible Doctors

Department	Person in charge	Position
Clinical Laboratories	Dr Phung Bich Thuy	Head of Laboratories
ICU	Dr Phan Huu Phuc	Vice head of dept
Infectious Diseases	Dr Nguyen Van Lam	Head of dept
Respiratory	Dr Dao Minh Tuan	Head of dept
Cardiovascular	Dr Nguyen Ly Thinh Truong/ Dr Cao Viet Tung	Vice head of dept
ER	Dr Le Ngoc Duy	Vice head of dept
NICU	A/Prof Khu Thi Khanh Dung	Head of dept
Coordinator	Ms Vu Thi Mai Anh	International Cooperation dept

ER: Emergency, ID: Infectious Diseases, NICU: Neonatal ICU

Topics

・日本大使館　Japanese Embassy

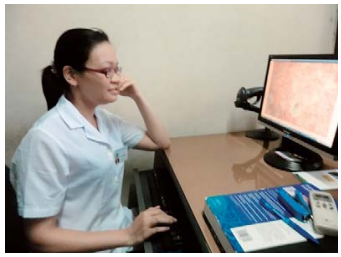
中井医務官 Dr. NakaiとDr. Duong

・JICA Vietnam

左から、Linh院長秘書、増田さん、藤田さん、鈴木所長、副院長、Dr. Phuc、Dr. Thuy、定本さん

・Pathology：Tapeworm

Diep先生に説明を受ける



単位授与
Certificate for students

国立小児病院(NCH)および研究所との単位互換(受講証)
国立ハノイ医科大学(HMU)との単位互換(受講証)

報 告

公衆衛生学実習「ベトナムでの感染症」

帝京大学医学部5年
○前原里美, 朝倉美貴子, 植木理子, 川島亮, 久保喜嗣, 古川元春, 吉武彰子

(1) ベトナムの環境

今回のベトナムでの公衆衛生学実習を通して、色々な国の文化が混じり合ったベトナムで、どのような医療が行なわれているのか、衛生環境や病院の設備等、色々なことを学ぶ事が出来た。到着してすぐ目の当たりにしたのがたくさんのモーターバイクが一般の車以上に走っていることと、多くの人々がそれらのモーターバイクに2人以上、もしくは子供を入れて4人など平気で乗せて走っている事だ。ベトナムに向かう前に読んだ本でベトナムの主な死亡原因は日本のようにNCDや癌などではなく、交通外傷だということが書いてあったのでこのような事情によるものだとすぐに理解することができた。

(2) ハノイ大使館で医系技官の方とのお話

2日目には、日本大使館にて医務官の中井呈子先生のお話を御伺いする事が出来た。ハノイには2年程前にいらっしゃったそうだ。医務官の主な仕事は現地の医療情報を収集して、外務省に提供する、現地に住んでいる在留邦人の診療、治療等を行なっているそうだ。ベトナムの場合、医務官は大使館にしか常駐していない為、3ヶ月に1回程度巡回しているそうだ。また、在留邦人、または旅行者の方がハノイの病院等で亡くなった場合、医務官が検死をすることになっている。ベトナムでは家で亡くなる方が多いため、人民委員会が死亡診断書を書いて提出するのだが、公安と法医学の技術や設備も備わっていないため、たとえ死亡原因が老衰だとしても死因不明と書いてあったりするそうだ。

(3) 国立小児病院の研究室にて

Thu Thi Bich Phung先生のお話を聞く事が出来た。学術振興会（JSPS）の招聘で、千葉大学大学院医学研究院にて鈴木和男教授の指導により博士号を取得したそうだ。ベトナムの感染症に対する研究はかなり進んでおり、主にその病院ではRT-PCRを使用して分子生物学の研究をされているそうだ。帝京大学をはじめ、オックスフォード大学、スウェーデンと共同研究を行っている。鈴木先生と共同研究したポスターも何枚も掲示してあった。Multiplex PCR、Simple PCR、Cap/CTM 48と3種類のPCRの機械があり、Multiplex PCRは25種類の細菌が同定できるそうだ。このような最先端の機械が数多くベトナムで使用されているという事実に、私は大変驚いた。いろいろな大学、有名な研究所と研究をされていらっしゃるNCHに、限りない将来性と、発展途上国ではなく、新興国であるという現実を目の当たりにした。

(4) 呼吸器科

Respiratory departmentは2週間前に移動した急造の病棟であり、10月になったら新病棟に移転するそうだ。外来

の患者は毎日65人位来ており、私たちは入院の患者さんを中心に見学した。呼吸器科の先生の話によれば、殆どの患者さんは肺炎が原因で入院されているそうだが、今回に限ってはベトナムでもめずらしいといわれているようなケースを見る事ができた。そのケースとは12歳の男性で、ハノイから少し離れた田舎町に住んでいる方だ。木から転落し、その後胸痛を自覚しこちらの病院に来院された。精査してみると、右肺に肺嚢胞ができており、肺切除して生検したところエキノコッカスであることが判明した。また、エキノコッカスの患者さんの祖母に当たる方に、お話を御聞きすることができ、動物との接触や生魚を食べる習慣があることから、経口虫卵摂取が考えられた。

（5）循環器内科

Cardiologyは50床あり、日本でいうPCCUが8床ある。1日5〜10床の患者さんが入れ替わるそうだ。また、2つ手術室を持ち、1日5例の手術を行う。日本では見られないような非常にめずらしい症例ばかりで、小児循環器学の豊田先生も国立成育医療センター以上かもとおっしゃられていた。NCHのCardiologyでは、HLHS 左心低形成症候群などに対してNorwood術などの高度な手術を行っていた。

（6）緊急救命室　ER

NCHでは医師不足が色々な科で目立っていたが、ERは特に不足していると思った。ERのスタッフはインターンを含め全15人おり、当直は月7〜8回と、非常に過酷な状況であることがわかった。また、医学生も戦力として考えられており、医学生の当直も月5回程あるそうだ。医学生でも虫垂炎などの手術を担当する事があり、医学生に対する教育は整っているとは言えない状況だった。ERに来る患者さんの疾患としては、虫垂炎、交通外傷、SAHなどの脳出血が多く、その他にも日本では聞いた事が無いような症例も多くあった。ベトナムを含めた東南アジアでは母親の教育があまり十分ではなく、非常に興味深い症例を見る事が出来た。生後8日の乳児が1日4回胎便することを知らず、下痢を止めるためにオピオイドを母親が投与しオピオイド中毒になったという症例だった。ベトナムの都市部では、カイザーが多く行われているそうだ。理由としては、自然分娩だと痛みを患うことが多く、ベトナム人女性の間ではカイザーの方が安全性も高く、合併症も少ないと信じられているからだ。ベトナムでは様々な面で教育不足が目立っている印象だった。

（7）新生児集中治療室　NICU

NICUは全4部屋で100名の患者さんがおり、3Fの100人の患者さんを合わせて、NICUで担当している。また、乳児死亡率最低の日本から来ているからか、感染症合併の未熟児の50%程度が亡くなるという事実に非常に驚きを感じた。また代謝性疾患が非常に多く、日本では先天性副腎過形成症、クレチン病、ガラクトース血症、フェニルケトン尿症などは早期発見のためマススクリーニングを義務付けているが、ベトナムでは自費診療となっており、早期発見できないケースが多いそうだ。自費で検査する場合は、OHG hospitalか日本の研究室に外注するとのことだった。また、*Elizabethkingia meningoseptica*という菌がよく髄膜炎を発症し、深刻な問題となっているそうだ。

（8）集中治療室　ICU

ICUの病床数は全部で70床で重症患者が40床、軽症患者が30床となっている。主に退院後のフォローは行なっていないそうだ。NCHのICU内の新病棟入院施設では、入り口があってすぐにDoctor's space、その奥に患者の病室がある。アメリカの入院設備を導入しており、日本の病院にはみられないものだそうだ（豊田先生談）。日本でも、沖縄中部病院では見られるそうだが、私もこのような入院施設はみたことがなかった。また、ベトナムは疫学という概念が全く発展しておらず、どの病気がどのくらいいるか、致死率はどれくらいかというような考えは通用しなさそうだった。今回ICUを回った際に非常に興味深かった症例が、12歳の男児でリケッチア感染症になった方だ。日本では見られないような非常にめずらしい感染症だが、ベトナムでは一年に何回かあるぐらい比較的多い疾患だそうだ。感染症病棟は全部で120床もあり、1階は50床、2階は70床であった。ただし1部屋4

床もあり、感染症病棟なのに換気等、特別な感染症対策を行なっているような設備は特になかった。その入院施設の部屋自体も日本と比較して非常に狭く、一つのベッドに2人の親子が座っている等日本では見られないような光景を目の当たりにした。また、冷房などの施設もないため、非常に蒸し暑く、他の施設と比べて快適な環境であるとはいえなかった。感染症病棟の特徴として、髄膜炎は髄膜炎専用の病室、咳主訴の方は咳の病室に分けられて一つの部屋に何人もの患者さんが入院しているということに非常に驚きを感じた。また、日本脳炎の患者さんが非常に多く、ワクチン摂取をしていないのか聞いてみたところ、ワクチン摂取を3回する機会をもうけてはいるが、ベトナムのお母さんたちはワクチンに対して抵抗性を持っており、摂取している子供は非常に少ない。日本脳炎自体はNCHだけでも年間100例とめずらしくなく、死亡率はそのうち5〜10%程度だそうだ。また、赤痢、チフスコレラ、大腸菌は非常によくみかけるが、経口薬剤をOTCから気軽に購入でき、簡単に治療できるため入院することは少ないと言っていた。

(9) ハノイ医科大学の講義「Infectious disease in Vietnam」

ハノイ医科大学の先生に、ベトナムにおける感染症の現状について講義をしていただいた。2012-2013年に麻疹ワクチンにより死亡する事故が起こり反対運動が起こってしまい、それゆえに2014年に大流行が起こってしまった。その時には31,313人が感染疑い、5,476人が確定診断され、146人が死亡した。現在ではワクチンの製造方法を変更し、ワクチン接種を勧奨している。コレラについては、2008年に再流行した。日本脳炎については、3回のワクチン接種の機会が設けられているにもかかわらず、主にハノイを含む北部において大流行している。理由としては、ワクチン接種が義務づけられていない、ワクチン自体の量が十分ではない、IgGなどの免疫の成立を確認しないことが原因ではないかと考えられている。最後に、耐性菌の問題についてお話ししてくださった。ベトナムでは耐性菌の問題が日本以上に深刻化しており、その理由としては、血液に接触する手技以外は手袋を着用せず処置するなど感染防御対策が未熟であること、抗菌薬が医師の処方箋が必要なくOTCで気軽に購入できることが挙げられる。日本では医師の抗菌薬に対する理解だけで耐性菌の問題を減らすことができるが、ベトナムでは法律での整備が必要となるということであった。今まで特に感染症に焦点を当てながら学んできたので、最後に体系的に学べたのはよい復習の機会となった。

○**Rimi Maehara**

On behalf of Students: Rimi Maehara, Mikiko Asakura, Michiko Ueki, Akira Kawashima, Yoshitsugu Kubo, Motoharu Furukawa, Shoko Yoshitake

(1) Environment of Vietnam

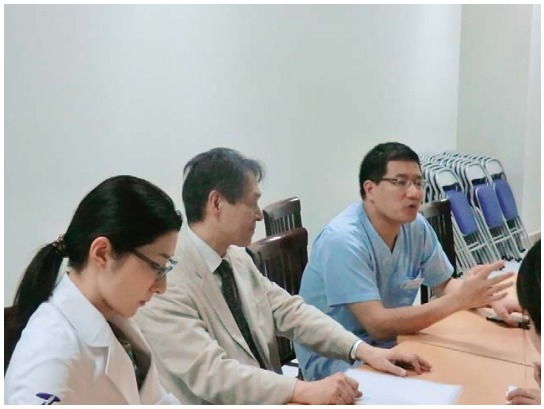
Through this practical training for public health in Vietnam that has blended cultures from various countries, I learned many things, including the way to provide medical care services, hygienic environment, and equipments in hospitals. The streets I traveled from the airport to the hotel after I arrived at Vietnam were especially impressive for me. At first glance, the streets near the airport looked fine and seemed to be kept in good condition. However, after I proceeded, I found the office buildings to be beautiful but were constructed incompletely and I also found residential areas in which even kitchens were visible from the entrance. Vietnam is an emerging country under economic development. It was interesting that I saw both developed and developing areas. I noticed immediately after arrival that more motorcycles are used than vehicles and two or more people (or with four people including children) ride on a motorcycle comfortably. In Vietnam, riding a motorcycle without wearing a helmet seems to be a common practice, which suggests that the traffic situation on public roads has not been developed yet. In a book I read before going to Vietnam, it was described that the main cause of death there is traffic injury and not non-communicable disease (NCD) and cancer as in Japan. After withessing the traffic situation, the reason for the main cause of death there became immediately clear for us.

(2) Talk with a medical technical officer at the embassy in Hanoi

On the second day, I visited the Japanese Embassy and met Dr. Teiko Nakai, a medical officer. She is a Third Councilor and was dispatched to Hanoi from the Ministry of Foreign Affairs approximately two years ago. According to her explanation, the main work as a medical officer involves collecting local medical information and providing it to the Ministry of Foreign Affairs; a medical officer also checks and treats Japanese residents living in the area. However, such medical care services are provided only within the embassy, as it is prohibited to carry out such services in the community based on reciprocal agreements for local physician qualifications. This would be considered a violation of the Medical Practitioner's Act. The embassy staff visits a local hospital where patients are treated. However, in Cuba, where Dr. Nakai worked, she treated patients within the embassy as few hospitals could provide medical services and economic barrier caused a lack of medical supplies. In Vietnam, childbirth has only happened twice at the embassy as most are performed at the French Hospital. Medical officers work primarily in developing countries and not in developed ones such as the United States and the United Kingdom. In the case of Vietnam, the Japanese Embassy in Hanoi is the only office where medical officers have a permanent position; however, Dr. Nakai visits other sites once every three months. In addition, when Japanese residents or tourists die in hospitals in Hanoi, as a medical officer, Dr. Nakai performs the autopsy. In Vietnam, many people die at home and the members of the commissariat prepare a certificate of death for submission. However, since the public safety commission, forensic specialists, and Ministry of Justice officers in charge of protecting Japanese living abroad are ill-equipped with few skills and supplies, they may describe the cause of death as unknown even if the cause of death is senility.

(3) Laboratory

At the laboratory, I was able to talk with Dr. Thu Thi Bich Phung who studied Ph.D. in Chiba University Graduate School of Medicine supervised by Prof. Suzuki. Studies on infection in Vietnam are considerably advanced. At the hospital, she conducts molecular biology research mainly using Reverse Transcription Polymerase Chain Reaction (RT-PCR). She has collaborated with Teikyo University, Kanazawa University, the Pasteur Institute, Oxford University, and Sweden researcher's group. Several posters indicating a collaborative research with Dr. Suzuki were displayed. There were three types of PCR devices (Multiplex PCR, Simple PCR, and Cap/CTM 48). She said 25 kinds of bacteria could be identified with the Multiplex PCR. According to her explanation, this device is expensive and used only for patients with sepsis who are admitted to ICU as it takes approximately JPY 500,000 per test. She said that the Simple RT-PCR, with which a great variety of disease sources could be identified including DNA viruses, RNA viruses, bacteria, mycoplasma, chlamydia, Legionella, and fungus, is often used in their research. Personally, I was very surprised that many of these cutting-edge devices have been used in Vietnam. Moreover, with my own eyes, I witnessed that studies have been performed with various universities and famous research institutes using such devices at the NCH. I thought NCH has boundless future possibilities and is located in not a developing country but an emerging one.



(4) Respiratory Department

Respiratory Department was moved and quickly constructed two weeks ago. It would be transferred to a new ward in October. There are 65 outpatients per day. We visited mainly the hospitalization area. According to a physician there, most patients are hospitalized due to pneumonia. However, we were able to observe a rare case in Vietnam at the time. The case is a 12-year-old boy living in the county far from Hanoi. This patient fell out from a tree. Afterward, having chest pain, he visited this hospital. Further evaluation revealed a pulmonary cyst located in the right lung. Lobectomy and biopsy were performed. Echinococcus was found as a result. We were able to talk with his grandmother and found that this patient had fed various animals including dogs, cats, 13 pigs, and three cattle. According to his physician's opinion, contact with the animals might be the cause. In addition, it was also supposed that eggs of the parasite might have been taken orally as the family regularly eats raw fish.

(5) Cardiology Division

There are 50 beds in the Cardiology Division and an additional eight

beds that are used for emergency care, referred to as Post Critical Care Unit (PCCU) in Japan. Five to10 patients rotate on these beds per day. There are two operating rooms and five operations per day. There are many rare cases that are not found in Japan. Dr. Toyoda in the Division of Pediatric Cardiology said that the number of these rare cases might exceed that of National Center for Child Health and Development. In the Cardiology Division of the National Children's Hospital (NCH), an advanced surgery, such as Norwood operation, was performed for patients with hypoplastic left heart syndrome (HLHS). The level of medical care in the cardiovascular disease division was even higher than any hospital in Japan. I heard that in Vietnam there are few hospitals in which advanced surgeries can be performed; hence, many patients visit only one hospital unlike in Japan.

(6) ER

A doctor shortage was noteworthy in various divisions at the NCH, especially situation is serious in the emergency room (ER). The number of staff in the ER was 15, including interns. It was found that doctors were on duty 7–8 times a month and that they worked under harsh conditions. There were five rooms for emergency visits. Five-member staff was in charge of the outpatient division from 16:00 to 22:00 and two from 22:00 to 8:00, whereas an average of 500 patients visit the hospital every day, and one physician had to be in charge of approximately 100 outpatients. Medical students were considered as members of the professional team and on duty approximately five times a month. Even medical students are also in charge of surgery for patients with, for example, appendicitis; thus, it cannot be said that the education for medical students is excellent. Patients who visit the ER commonly have appendicitis, traffic injuries, and cerebral hemorrhages, such as subarachnoid hemorrhage (SAH). There were also many cases that we have never heard of in Japan. For intoxication, common cases include lead poisoning that is mainly found in traditional medicine, accidental ingestion of detergent which was in a plastic bottle, chemical pneumonitis caused by oil, and 90% alcohol (by drinking milk right after disinfecting the nose and face). In Southeast Asia including Vietnam, education to mothers is not provided adequately and we were able to observe interesting cases. For example, not knowing that meconium occurs in infants four times a day, a mother gave opioids to her infant on the eighth day of life to stop diarrhea and her infant developed opioid intoxication. Moreover, she did not confide what kind of drug was used since the use of opioids is illegal. This case is interesting and seems to be rare in Japan. However, such cases are relatively common in Vietnam; it is impressive that even the physicians said this case was difficult to handle. I heard that cesarean section is frequently performed in urban areas of Vietnam, because Vietnamese women believe that cesarean section is safer and associated with fewer complications than natural childbirth, which commonly causes pain. Actually, it is the reverse, and physicians have recommended natural childbirth while many Vietnamese women tend to avoid it. I was impressed with the lack of education in various aspects in Vietnam.

(7) NICU

There were a total of 100 patients in four rooms in the neonatal intensive care unit (NICU). The staff in NICU was in charge of these patients plus 100 patients in the rooms on the third floor. Perhaps since we came from Japan with the lowest infant mortality, we were surprised by the fact that around 50% of premature infants with combined infection died. In addition, there were many cases of metabolic disorders in Vietnam. In Japan, it is mandatory to perform mass screening for the purpose of early detection of congenital adrenal hyperplasia, cretinism, galactosemia, and phenylketonuria. On the other haud, in Vietnam, these screenings are performed at the patient's own expense; therefore, many such cases could not be detected early. It is said that a screening performed at a patient's own expense are outsourced to OHG hospitals or a laboratory in Japan. A concept such as mass screening seems not to have yet penetrated far into Southeast Asia. In addition, the bacteria called *Elizabethkingia meningoseptica* frequently causes meningitis, and it is becoming a serious problem in Vietnam.

(8) ICU

The hospital bed capacity of the ICU was 70 beds in total (40 beds for severely affected patients and 30 for mildly ill patients). No follow-up after discharge was performed. In the new ward for hospitalization in the ICU of the NCH, there were patient rooms in the depths of "Doctor's space" near the entrance. In this "Doctor's space," preparations necessary for infection prevention and prior to various surgical operations can be performed. (According to Dr. Toyoda) This system was based on the hospital equipment in the U.S. and is not found in hospitals in Japan. In Japan, this system can be found in Okinawa Prefectural Chubu Hospital, which is a famous navy hospital. However, I have not seen this kind of inpatient facility. In Vietnam, the concept of epidemiology was not developed at all, and concepts such as how much of which diseases are there and what is their mortality were not widely accepted. As a possible explanation, to even examine a fatality rate, many people wait for death at home without even thinking of receiving palliative care and end-of-life treatment; hence, it was not possible to grasp the number of death accurately at the hospital. The most interesting case in our visit was a 12-year-old boy who had rickettsial

infection. It is an extremely rare infection that is not found in Japan. However, this disease is relatively common and occurs several times per year in Vietnam. This boy had atypical symptoms that even the physicians in the ICU had never seen. It was impressive that they found the diagnosis especially difficult. When we visited the ICU, I was surprised that they did not wear gloves when being in contact with blood (e.g., blood sampling) in Vietnam. In contrast, in Japan, whenever we have contact with patients, we are mandated to wear gloves at all times. There were as many as 120 beds in total in the infection isolation nursing unit (50 beds on the ground floor and 70 on the first floor). There were four beds in one room. Although it was an infection isolation nursing unit, there was no special equipment for infection control such as ventilation. The room in the inpatient facility was much smaller than Japan. We witnessed a rare scene that two parents and a child sat on one bed, which is never seen in Japan. In addition, it was awfully hot and humid as there was no equipment such as an air conditioner; it was not comfortable than other facilities. As for the characteristics of the unit, patient rooms were divided according to disease (e.g., patients with cerebral meningitis were admitted to cerebral meningitis-specific rooms; those with the chief complaint of a cough were admitted to cough-specific ones) to house many patients in one room; this situation surprised us. Too many patients had Japanese encephalitis. We asked whether they receive vaccination. Their responses was as follows: although they prepare three opportunities for vaccination, Vietnamese mothers have resisted vaccination; consequently, only a few children actually receive it. Since education for mothers is not widespread, they do not know which vaccines have been administered in the first place and they often lose the record that have been distributed. They say that Japanese encephalitis in itself is not a rare disease and occurs in 100 patients a year, with a mortality rate of around 5%–10% in NCH. We asked about other infections in this unit. Their responses were as follows: dysentery, typhoid, cholera, and *Escherichia coli* were common, but hospitalization was rarely required as oral, over-the-counter drugs (OTCs) were available and they could be treated easily.

(9) Lecture at Hanoi Medical University; "Infectious disease in Vietnam"

The professor at the Hanoi Medical University lectured on the current situation of infectious diseases in Vietnam. In 2012–2013, an accidental death from live attenuated measles vaccine occurred, causing an opposition movement against vaccination and leading to an outbreak in 2014. At that time, there were 31,313 people suspected of infection, 5,476 of those were diagnosed definitively, and 146 died. The manufacturing method of the vaccine was changed. Currently, vaccination is recommended. Cholera re-emerged in 2008. Although three opportunities for vaccination have been prepared, Japanese encephalitis outbreaks occur in the northern part of the country, including Hanoi. The reason of this situation are thought to be as follows: vaccination is not mandatory; the quantity of vaccine is inadequate; and establishment of immunity (e.g., IgG) is not confirmed. At the end of the lecture, the professor talked about the problem of resistant bacteria. In Vietnam, this problem is worse than Japan as infection prevention is poor (e.g., treat patients without gloves unless medical staff come in contact with blood) and antimicrobials are readily available as OTC without prescription. In Japan, the problem of resistant bacteria can be reduced by simply teaching physicians antimicrobials. However, in Vietnam, it is said that the development of laws is required to solve this problem. I have learned about various issues focusing on infection, so in the end systematic learning provided me with a good opportunity for review of what I learned.

50th Anniversary of Teikyo University ADC International Symposium 「Infectious Diseases and Host Defense（感染症と生体防御）」

日時：2016年9月16日（金）13：00-18：00
会場：臨床大講堂

帝京大学創立50周年記念国際会議の一環として、ADC国際シンポジウム「Infectious Diseases and Host Defense」を開催しました。海外からは、ベトナムの百日咳の臨床像：Tran Minh Dien准教授（VNCH副院長）、Chikungunya Virusesとワクチン：Pratima Ray教授（インド Jamia Hamdard大学）、インフルエンザ：Stavros Selemidis博士（オーストラリア Monash大学）の3名の先生方に講演いただき、国内からは、天然痘撲滅：倉田毅教授（元国立感染研所長、国際医療福祉大学）、寄生虫ミトコンドリアの多様性ー薬剤標的として：北 潔教授（長崎大学）、グローバル化における結核研究：加藤誠也副所長（結核研究所）、ウイルス性胃腸炎の分子疫学と病態：牛島廣治教授（日本大学）の4人の先生方に現状と問題点をお話しいただき、グローバルな視点で感染症と生体防御・ワクチンについて討論できました。準備していました100名分の要旨集・ハンドアウトを急きょ増す刷りするなど予想以上の119名（内、海外13名）の方に参加していただきました。

Program

Organizer: Prof. Kazuo Suzuki, Director of Asia International Infectious Disease Contol (ADC), Teikyo University

Session I Infectious Diseases

Clinical characteristics of pertussis's patients in the Vietnam National Hospital of Pediatrics, in 2015
Associate Prof. Tran Minh Dien, Vice Director of the National Children's Hospital, and Hanoi Medical University, Hanoi, Vietnam
Chair Dr. Nguyen Huu Tu, Kansai Medical University, Osaka, Japan

Smallpox eradication
Prof. Takeshi Kurata, International University of Health and Welfare & NIID, Former Director-General, Japan
Chair Visiting Prof. Shoji Kawachi, ADC, Teikyo University, Tokyo, and Tomakomai City Hospital, Tomakomai, Japan

Diversity of parasite mitochondria -as drug targets-
Prof. Kiyoshi Kita, Nagasaki University, School of Tropical Medicine and Global Health, Nagasaki, Japan
Chair Prof. Tomoko Yamamoto, ADC, Teikyo University, Tokyo, Japan

Tuberculosis research agenda in the context of globalization
Dr. Seiya Kato, Vice Director, Research Institute of Tuberculosis, Tokyo, Japan
Chair Visiting Prof. Kiyoko Akagawa, Kitasato Institute, Tokyo, Japan

Session II Host Defense and Vaccination

Regulation of Influenza A Virus Pathogenicity by NADPH Oxidases
Dr. Stavros Selemidis, Department of Pharmacology, Monash University, Clayton, Australia.
Chair Prof. Yasuo Ono, Teikyo University, Tokyo, Japan

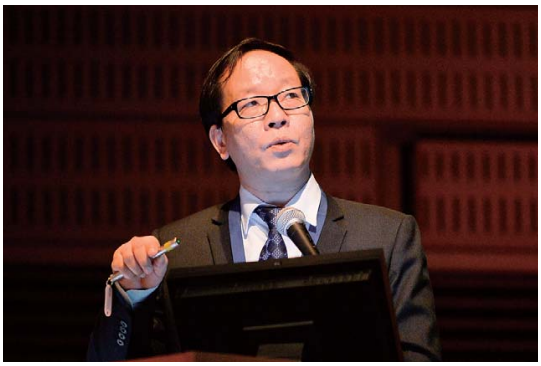
Human Immunity to Chikungunya Viruses: Prospects for Vaccine
Prof. Pratima Ray, Department of Biotechnology, Faculty of Science, Jamia Hamdard University, New Deli, India
Chair Prof. Koichi Makimura, Teikyo University, Tokyo, Japan

Molecular epidemiology and pathogenesis of virus gastroenteritis
Prof. Hiroshi Ushijima, Department of Microbiology, Nippon University School of Medicine, Tokyo, Japan
Chair Prof. Masakazu Mimaki, Teikyo University, Tokyo, Japan

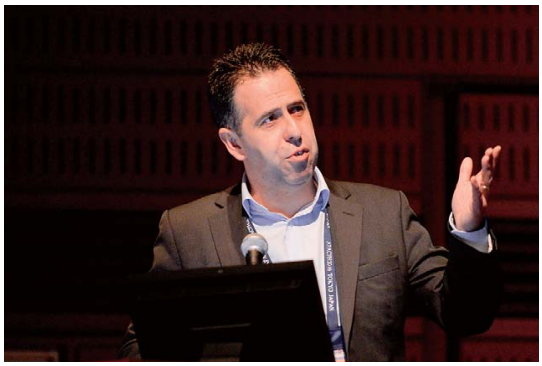
【シンポジウムの様子】

開会あいさつ
鈴木和男（ADC所長）
Opening: Prof. Kazuo Suzuki

グローバル化における結核研究
加藤誠也副所長（結核研究所）
Tuberculosis: Dr. Seiya Kato



ベトナムの百日咳の臨床像
Tran Minh Dien准教授（VNCH副院長）
Pertussis's patients: Asscciate Prof. Tran Minh Dien



インフルエンザ
Stavros Selemidis博士（オーストラリア　Monash大学）
Flu A Pathogenicity: Dr. Stavros Selemidis

天然痘撲滅
倉田　毅教授（元国立感染研所長、国際医療福祉大学）
Smallpox eradication: Prof. Takeshi Kurata

チクングニアウイルスとワクチン
Pratima Ray教授（インド　Jamia Hamdard大学）
Chikungunya Viruses: Prof. Pratima Ray

寄生虫ミトコンドリアの多様性ー薬剤標的として
北　潔教授（長崎大学）
Diversity of parasite mitochondria: Prof. Kiyoshi Kita

ウイルス性胃腸炎の分子疫学と病態
牛島廣治教授（日本大学）
Gastroenteritis Virus: Prof. Hiroshi Ushijima

Joint Meeting of e-ASIA: Japan-Vietnam-Philippines at Taal Vista Hotel, Philippines

October 17th-18th, 2016

3年目を迎えたe-ASIA project合同会議を2016年10月17, 18日にフィリピンにて開催
Project Meeting for e-ASIA Joint Research Program was Held on October 17th-18th in Philippines
研究開発課題名：日本・ベトナム・フィリピンでの疫学調査によるインフルエンザ・結核による呼吸器感染症の3か国比較
International Study on Pulmonary Disease Infected with Influenza Virus and Mycobacterium Tuberculosis

研究開発代表者 Project Leader：鈴木和男（帝京大学アジア国際感染症制御研究所）
相手国研究代表者 Local Leaders：Dr. Phung Thi Bich Thuy in the National Children's Hospital, Vietnam and Prof. Jamie Montoya, University of the Philippines College of Medicine

All Attendee in Joint Meeting of e-ASIA at Taal Vista Hotel in Philippines

Flu Group Meeting of e-ASIA at RITM in Philippines

General Meeting of e-ASIA at Taal Vista Hotel in Philippines

プロジェクトの状況報告
Progress Reports

1）インフルエンザ研究チーム
遺伝子変異による重症化の機構解析（日本)、インフルエンザの臨床（ベトナム、フィリピン）
2）結核研究チーム
次世代シークエンサーを用いて、特異的な EAI 株の遺伝子構造が、従来の結核菌分類法の落とし穴と遺伝子型決定のエラーを引き起こす原因になる可能性があることを示した。ベトナム：176 名の塗抹陽性結核患者を登録、フィリピンでは192名が結核菌として同定された。東南アジア地域の結核菌の特徴を突き止めていく必要がある。

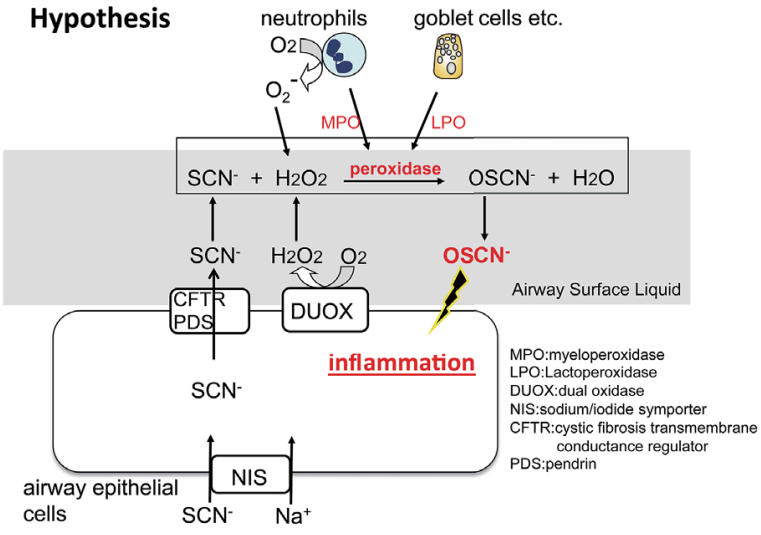
1）Influenza Research Team
Japanese group: A role of Non-structural protein 1 (NS1) for severe pneumoniae induction using modeling simulation in gene mutation in influenza virus.
Vietnam and Philippines group: Swab spacemen collection for analysis of viral mutations in Vietnam. In Philippines the protocol approval by the FDA and preparation of protocols for a clinical trial for intervention with Leucomycin-Josamycin for patients with severe pneumoniae.
2）Tuberculosis Research Team
In Vietnam, 176 sputum-smear positive active pulmonary TB patients have been recruited. In the Philippines, 192 were culture-proven. There is a need for characterizing these MTB strains in Southeast Asia.

Report-1

ヒポチオシアン酸による気道炎症反応

帝京大学 ADC 研　講師　**鈴木章一**

2016年10月17, 18日にjoint meeting of e-ASIAに参加した。この会議において、私は、ミエロペルオキシダーゼ（MPO）やラクトペルオキシダーゼ（LPO）の酵素反応産物であるヒポチオシアン酸（$OSCN^-$）で気道上皮細胞を刺激すると、炎症反応において中心的な役割を担う転写因子であるNF-κBが活性化されることを報告し、インフルエンザウイルス感染時における炎症反応に関する新規仮説を提唱した。気道上皮細胞を覆うairway surface liquidにはCFTR等の陰イオントランスポーターの働きにより、高濃度のチオシアネートイオン（SCN^-）が存在する。また、インフルエンザウイルスが感染した際には、気道上皮細胞のDUOXや好中球のNOX2により、過酸化水素（H_2O_2）が産生されることも知られている。従って、図に示したように、SCN^-は杯細胞から分泌されたLPOや好中球から放出されたMPOの酵素反応により$OSCN^-$へと変換されうる。私共は、インフルエンザウイルスが感染すると、$OSCN^-$が産生されて気道上皮細胞のNF-κBが活性化され、IL-6 やIL-8等の炎症性サイトカインの産生が促進することによって炎症反応が増幅するのではないかと考えている。



Airway Inflammation Induced by Hypothiocyanite ($OSCN^-$)

Associate Professor, Shoichi Suzuki Ph.D.
ADC, Teikyo University

On 17-18th October, 2016, I participated in the joint meeting of e-ASIA. In this meeting, I reported an in vitro data that hypothiocyanite activates NF-κB. NF-κB is a key transcription factor for amplifying the in-

flammatory response in airway epithelial cells. I also presented a novel model of inflammatory response induced by influenza virus infection. As shown in the figure in the previous page, the airway surface liquid (ASL) on airway epithelial cells is known to contain a large amount of thiocyanate (SCN^-), because it is actively transported into ASL through several anion transporters such as CFTR and pendrin. It is also known that influenza virus infection causes the production of hydrogen peroxide (H_2O_2) by DUOX and NOX2, from the epithelial cells and neutrophils, respectively. Therefore, it is possible that SCN^- is converted into hypothiocyanite ($OSCN^-$) by lactoperoxidase (LPO) and myeroperoxidase (MPO) derived from goblet cells and neutrophils, respectively. We hypothesize that during the influenza virus infection, $OSCN^-$ is generated by the peroxidases, and induces inflammatory cytokines such as IL-6 and IL-8 through NF-κB activation in the epithelial cells, resulting in amplifying inflammatory response.

Report-2

新規抗インフルエンザ薬の開発と臨床研究への道筋

帝京大学 ADC 研・感染症研究室　助教　菅又龍一

帝京大学ADC研究所は、現在、日本-フィリピン-ベトナムとの3カ国間において、脅威となる感染症に対する制御を目的としたe-ASIAプロジェクトを推進している。当プロジェクトにおける私の役割は、インフルエンザ感染症をはじめとする病原性重篤肺炎に対する新規治療薬の開発あるいはリポジショニングを目指し、アジアを中心とした感染症に対する治療法の基盤を構築することである。

去る2016年10月17、18日、我々のプロジェクトグループはフィリピンで開催された本プロジェクトの国際報告会議に出席し、研究データを報告した。私は、本来抗菌作用を示すマクロライド系薬剤の一つであるロイコマイシン（Leucomycin A_3）が、ヒト細胞培養系においてインフルエンザA/H1N1ウイルスの増殖を抑制すること、また、動物実験においても致死濃度のウイルスで感染させたマウスの肺炎を抑制しつつ、生存率を著しく上昇させる（>80%）ことを示した研究データを発表した。

ロイコマイシンはすでにヒトへの安全性が確かめられ、薬剤として承認されている背景があるため、フィリピンのプロジェクトチームと連携し、同国におけるインフルエンザを含めた感染性重篤肺炎に対するロイコマイシンを用いた臨床試験を進める方向で調整に入ることが、会議の場で約束された。我々が推進してきた研究の成果が、当該プロジェクトを通じて国際的観点から感染症の制御に実を結ぶ可能性が期待される会議であった。

Development of Novel Anti-influenza Virus Drugs and Direction for Clinical Studies

Ryuichi Sugamata Ph.D.
ADC, Teikyo University

ADC institution is actively promoting e-ASIA project. The project is international collaboration for prevention/regulation of serious epidemics among Japan, Philippines and Vietnam. In this project, my responsibility is a development and repositioning of novel therapeutic drugs for the treatment of pathogenic severe pneumonia including influenza, and establishment of treatment approaches against infectious diseases with a central focus on Asian countries.

Project members from ADC institution attended at regular conference in Philippines on October 17-18, 2016. During the international meeting, I reported our research data: a leucomycin A_3 macrolide antibiotic played an inhibitory role in viral propagation of influenza A/H1N1 in human cellular culturing system and the leucomycin A_3 lead to remarkable survival advantage of mice which were lethally-infected with the A/H1N1 virus.

The leucomycin A_3 has already been assessed its safety for human body and has approved as the drug for human patients. Based on this advantage, cooperating with Philippines team, the clinical trial/research by use of leucomycin A_3 for human patients whose case are severe pathogenic pneumonia including influenza were authorized in the conference of e-ASIA project.

The international conference was fruitful opportunity for us. Our research accomplishments have promising possibility for establishment of prevention and regulation of infectious diseases.

Molecular Structure in Gene Mutation of Neuraminidase of Influenza Virus Type B Isolated from Swab of Patients Showing Fever Duration

Haruka Hishiki[1], Yosuke Kameoka[2], Yusuke Kato[3,4], Reiko Itoh[5], Tomohiro Someya[6], Nobue Inoue[7], Mana Haraki[8], Tomomichi Kurosaki[9], Shoichi Suzuki[5], Tomoko Ogawa[10], Naruhiko Ishiwada[11]*, and Kazuo Suzuki[5,12]

[1]Department of Pediatrics, Chiba Univesity Graduate School of Medicine, Inohana 1-8-1, Chuoku, Chiba city, Chiba, 260-8670, JAPAN
[2]Department of Research and Development, A-CLIP Institute, Inohana 1-8-15, Chyuoku, Chiba city, Chiba, 260-0856, JAPAN
[3]Institute for Health Sciences, Tokushima Bunri University, 180 Nishihama, Yamashiro-Cho, Tokushima 770-8514, JAPAN
[4]Institute for Enzyme Research, Tokushima University, 3-18-15 Kuramoto-Cho, Tokushima 770-8503, JAPAN
[5]Asia International Institute of Infectious Diseases Control, Teikyo University, Kaga 2-11-1 Itabashi-ku, Tokyo 193-8605, JAPAN
[6]Department of Pediatrics, Goi Hospital, 5155 Goi, Ichihara city, Chiba, 290-0056, JAPAN
[7]Department of Pediatrics, Yamanouchi Hospital, 3 Machiho, Mobara city, Chiba, 297-002, JAPAN
[8]Mana Children's Clinic, 7-9-2 Chuou, Oyumino, Midoriku, Chiba city, Chiba, 266-0032, JAPAN
[9]Kurosaki Children's Clinic, 6-23-6 Konakadai, Inageku, Chiba city, Chiba, 263-0043, JAPAN
[10]Chiba Prefectural Institute of Public Health, 666-2 Nitonachou, Chuoku, Chiba city, Chiba, 260-0801, JAPAN
[11]Department of Infectious Diseases, Medical Mycology Research Center, 1-8-1 Inohana, Chuoku, Chiba city Chiba, 260-8673, JAPAN
[12]Department of Immunology, National Institute of Infectious Diseases, Tokyo 162-8640, JAPAN

Corresponding author (*)
Naruhiko Ishiwada
Department of Infectious Diseases, Medical Mycology Research Center, 1-8-1 Inohana, Chuoku, Chiba city, Chiba, 260-8673, JAPAN
E-mail: ishiwada@faculty.chiba-u.jp

Received Nov 30, 2016; **Revised** Dec 22, 2016; **Accepted** Dec 22, 2016

Abstract

Purpose **Neuraminidase inhibitors have been used for the treatment of both influenza A and B viral infections in children. Influenza viral infection occasionally shows prolonged duration of the fever despite the use of neuraminidase inhibitors, suggesting drug resistance. It has been reported that mutations of the viruses are related with the drug resistance. Here, we studied association between prolonged fever and mutations in influenza B.**

Methods **RNA was isolated from fixed nasopharyngeal swab of 206 patients with influenza in 2013/2014 season. The samples from ten patients having fever over 38˚C over 48hr were analyzed for neuraminidase sequences. Structural models reflecting the gene mutation of neuraminidase of influenza B virus (BNA) were analyzed.**

Results **Patients infected with influenza B showed longer duration of fever on average than those with influenza A. Sequences of BNAs from the patients with prolonged fever were different from that of the strain for vaccine (B/Massachusetts/02/2012). Two of the sequences in the samples M2-1 and K41-1 contained mutations for potential drug resistance: S99N, T106I, K125T and S295R in the sample M2-1 and I262M, V271T, K/E272Q, E320K, D342G and M375K in the K41-1. Structural models of BNA suggested that R295 of the M2-1 and Q272 and K375 of the K41-1 may be responsible for the drug resistance.**

Conclusions **Drug-resistant mechanism of BNA was clarified by using structural model analysis. Proper use of neuraminidase inhibitors against influenza virus infection is necessary in clinical setting.**

Key words: Influenza virus, Type B, drug resistance, children, neuraminidase inhibitor

Introduction

Influenza viral infection is prevalent every winter season in Japan. Influenza virus induces respiratory tract infections in various age groups, from children to elderly persons. For the treatment of influenza viral infection, neuraminidase inhibitors such as oseltamivir, zanamivir and laninamivir were developed and widely used in ambulatory settings. Neuraminidase inhibitors had the effect of shortening duration of febrile period of the patients with influenza viral infection[1)]. On the other hand, some of the cases with influenza viral infection showed prolonged duration of the fever despite the use of oseltamivir. The percentages of these cases were much higher with influenza B viral infection than influenza A infection[2,3)]. The precise mechanism of this phenomenon has not been clarified. In Japan, one influenza B strain with reduced drug sensitivity possessing a Gly402Ser neuraminidase substitution was isolated from a child who had received oseltamivir and seven influenza B strains with reduced sensitivity carrying other mutations were isolated from untreated children, during the 2004-2005 influenza season[4)]. However, according to the data from patients with influenza B infection from the 5 years (2009-2013) of the international prospective Influenza Resistance Information

Study, phenotypic resistance to oseltamivir or zanamivir was not found in B/Victoria and B/Yamagata viruses[5].

In the present study we collected influenza B virus genome isolated from nasopharyngeal swab of the children who had prolonged fever more than 48 hours after the treatment of neuraminidase inhibitors during the influenza B viral epidemic in 2013/2014 season. Using structural models and gene mutations of neuraminidase of influenza B virus, we analyzed reasons for prolonged fever of the patients.

Methods

Sampling from patients with influenza

1) Isolation of swab from patients

During the influenza epidemic period from 18th February to 8th April, 2014, we collected samples from the 206 pediatric patients less than 16 year of age with influenza viral infection at four outpatient clinics in Chiba prefecture, Japan. All patients had fever and respiratory symptom and were diagnosed influenza by using rapid antigen detection kit. We gave the written consent of the study to the patients and/or their guardians. The clinicians prescribed neuraminidase inhibitor for the patient after diagnosis. Age, sex, onset date of the fever over 37.5 degree and influenza vaccination status in 2013/2014 season were investigated at the time of first visit of the clinics. The decision of additional medication except neuraminidase inhibitor for the patients depended on the clinicians. The compliance of neuraminidase inhibitor, the clinical symptom and clinical course after treatment, side effect of neuraminidase inhibitor were investigated at the time of second visit of the clinics.

2) Viral typing A or B with rapid influenza antigen detection kit

Rapid influenza virus antigen detection kit (immune-chromatography) that was used was adopted by each clinic. Nasopharyngeal swabs were used as the materials. The kit was used at the bedside and the result was informed to the patients and their guardians by clinicians. After using antigen kit, remaining materials were stocked in a refrigerator.

3) Approval of Ethical Committee

This study was approved as 1749 on August 29, 2014 in Chiba University and Teirin 14-136 on Dec 4, 2014 in Teikyo University.

Isolation of viral RNA

The swab fluid was fixed with 500 µl of a fixer (A-CLIP Institute, Chiba, Japan) and then stored at 4˚C. Viral RNA was extracted from an aliquot of residual fluid from a commercial clinical diagnostic kit. Three drops of the residual fluid were put into 0.5 ml of the viral RNA retaining solution (A-CLIP Institute, Chiba, Japan). Viral RNA retaining solution was applied to the mini column of QIAamp viral RNA mini-kit (Qiagen, Valencia, CA) as described in manufactures instruction.

Measurement of viral typing

Viral typing was performed by M protein of influenza virus type A and hemagglutinin (HA) for type B with a real-time PCR. Precise viral typing was performed after the study period. The result of rapid antigen detection kit was confirmed by the diagnosis in PCR analysis. Type A and B subtype analysis was done basing on PCR method.

Quantitative real-time PCR and Reverse transcription-PCR analyses

Total RNA extracted from the fixed swab stored at 4˚C was treated with DNase (TURBO DNA-*free*; Ambion, Austin, Texas) and used as a template to synthesize complementary DNA (cDNA) with the Superscript VILO cDNA Synthesis Kit (Invitrogen). Using specific primer sets (Supplemental Table 1), the expression of each gene was quantitated with SYBR Green PCR Master Mix (Applied Biosystems, Foster City, California) by the StepOne Real-Time PCR System (Applied Biosystems). Reaction conditions were 95˚C for 15 seconds and 60˚C for 1 minute, repeated for 40 cycles, with hot start at 95˚C for 10 minutes. Each PCR analysis was run in duplicate for each sample.

Gene sequences in neuraminidase region of cDNA from viral RNA:

Reverse transcription-PCR (RT-PCR) and sequence analysis

Isolated viral RNA neuraminidase gene was amplified by Reverse transcription-PCR (RT-PCR) using a SuperScript III One-Step RT-PCR kit (Invitrogen, Carlsbad, CA). Amplification primer sets and their covering regions were as described in Table 1 and Fig. 1, respectively.

Neuraminidase sequences of 10 viruses tested in this study were deposited into the GenBank database. Base positions of primers for neuraminidase gene are given in B/CHIBA/9/2014 (Global Initiative on Sharing Avian Influenza Data (GISAID) Isolate ID EPI_ISL_163854) Standard influenza virus B Yamagata and Victoria were supplied from Dr. Ogawa in Chiba Institute of Health, Chiba City.

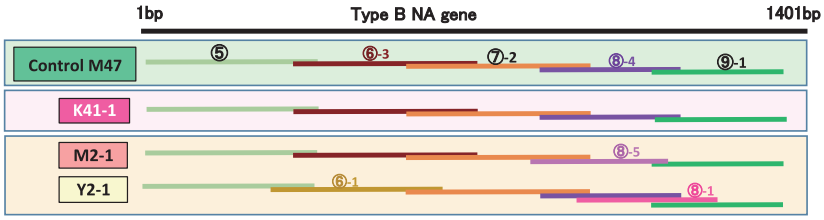
Molecular modeling of neuraminidase region of type B influenza (BNA)

Alignment of BNA sequences was performed with Clustal W. Swiss MODEL[6] was implemented to model the structures of BNAs from K41-1 and M2-1 strains. Structural analysis and presentation were performed using by Swiss PDB Viewer[6].

Table 1. Primer sets for cDNA amplification

Primer set No.	Start base of forward primer	Forward primer	End base of reverse primer	Reverse primer	Accession No
Set 5	236	AAGGGGTGACACTTCTTCTC	532	CTACTGTTGGGATTTTGCCC	CY019517.1
Set6-1	430	CATTATGCAGCCCAACCAG	769	CTGAAGCTGAGCCATCAGT	CY019517.1
Set6-3	490	GGGCAAAATCCCAACAGTAGAAA	801	ATTTCTTTTATTATTCGGCCCTCTCG	CY019517.1
Set7-2	678	CACAAGAAAGTGCCTGCAA	969	TCTGGGGGTGTCCAAATAAG	CY019517.1
Set8-1	949	GTGGAGACTGATACAGCAGA	1254	CCAACCAGGTTCTTCCATTG	Non
Set8-4	864	CCATAGAATGTGCCTGTAGAGA	1168	TGGGTCTCCATCATACTTTACATACAG	Non
Set8-5	832	GGAATGCACATGCGGATTTGC	1117	AGACATCGTCCGAGAGTACCA	Non
Set9-1	1100	TACTCTCGGACGATGTCTAAAACTAAA	1357	CCCATTAAACAGTAAATGGCTGT	Non

Fig. 1. Primer sets for BNA sequence



Results

Clinical evidences

We investigated the clinical symptoms of 206 pediatric patients with influenza virus infection and compared oseltamivir-treated, zanamivir-treated, and laninamivir-treated groups in this research period. Among 206 patients, oseltamivir-treated group, zanamivir-treated group, laninamivir-treated group was 99 patients, 83 patients, and 24 patients, respectively. Concerning the average onset of age, oseltamivir-treated group, zanamivir-treated group, laninamivir-treated group was 8.9 year of age, 9.2 year of age, and 5.3 year of age, respectively. The rate of influenza vaccination status was 45.5% in oseltamivir-treated group, 55.4% of zanamivir-treated group and 54.2% of laninamivir-treated group. The drug compliance of each neuraminidase inhibitor was good in all three groups. The duration of fever after administration of the first dose of each neuraminidase inhibitor was significantly prolonged in the patients with influenza B infection (average 43.1 hours) than in the patients with influenza A infection (average 31.8 hours), although there were no statistically significant difference in the clinical efficacy and the side effect among three groups. The number of biphasic fever episodes in patients treated with neuraminidase inhibitors was rare (two episodes of oseltamivir-treated group and one episode of zanamivir-treated group). Under the good drug compliance, all three neuraminidase inhibitors showed same efficacy for the treatment of influenza virus infection in children.

Subtype of Type A and Type B

Analyzed type of virus showed that 9 patients were positive with type A, including 3 patients with A/H3N2 and 6 with A/H1pdm09. In addition, 53 patients were estimated to be positive with type B according to the results from antigen kits. Among the patients with type B, 15 patients had high fever, which was more than 38˚C, for less than 48 hours, whereas 38 patients with type B had high fever for more than 48 hours. 28 of the 38 patients with prolonged fever were subject to one time sampling for real-time PCR, whereas 10 of those were subject to two times sampling. 23 of the 28 patients with one time sampling were positive with only Yamagata subtype of the B type, whereas the rest of the 28 was doubly positive with Yamagata and Victoria subtypes. 20 samples from 10 patients with two times sampling included those with 13 positive with only Yamagata subtype, 4 doubly positive and 3 negative results with real time PCR. All of the 3 negative results were from the samples at the second visits to the hospital.

Differences in BNA sequences from that of annual vaccine

The first swabs taken of 10 patients who had high fever over 38˚C over 48hr and were subjected twice were analyzed for BNA sequences. Sequencing of three samples called K41-1, M2-1 and Y2-1 of the 10 subjects successfully completed as well as that of the M47 sample from a patient without prolonged fever. Table 2 indicates the comparison of the sequences of these samples with that of BNA of B/Massachusetts/02/2012, one of the Yamagata subtypes from which annual vaccine was produced. All the samples from the patients with prolonged and non-prolonged fever showed substitutions compared with B/Massachusetts/02/2012.

Identification of novel mutations for drug resistance

We identified novel mutations for drug resistance to oseltamivir and zanamivir in the K41-1 and M2-1 strains, respectively

Table 2. Amino acid substitution due to mutation of BNA gene

Base position	Source from Patients				AA position	Source from Patients			
	M47	K41-1	Y2-1	M2-1		M47	K41-1	Y2-1	M2-1
Fever time:	19hr (Control)	95hr	10hr	68hr	Fever time:	19hr (Control)	95hr	10hr	68hr
Drug:	Zanavir	Oselamivir	Zanavir	Zanavir	Drug:	Zanavir	Oselamivir	Zanavir	Zanavir
296				296G/A	99				S99N
317	317T/C	317T/C	317T/C		106	I106T	I106T	I106T	
374	374C/A	374C/A	374C/A		125	T125K	T125K	T125K	
443		443A/G			148		E148G		
557	557A/G		557A/G		186	K186R		K186R	
593		593G/A			198		S198N		
657		657A/T			219		K219N		
703		703G/A			235		D235N		
730		730C/T			244		P244S		
742	742G/A		742G/A		248	V248I		V248I	
786		786A/G			262		I262M		
811		811G/A			271		V271T		
812		812T/C							
814		814A/C			272		K272Q		
883	883C/A	883C/A	883C/A		295	R295S	R295S	R295S	
958	958G/A	958G/A	958G/A		320	E320K	E320K	E320K	
985		985A/G			329		N329D		
1018		1018G/A			340		D340N		
1025		1025A/G			342		D342G		
1027	1027A/G		1027A/G		343	K343E		K343E	
1117		1117A/G			373		K373E		
1124		1124T/A			375		M375K		
1165		1165A/G			389		T389A		
1176		1176A/T			392		E392D		
1183		1183G/A			395		A395T		
1186		1186C/T			396		L396F		

The samples, K41-1, Y2-1 and M2-1, are those from patients with prolonged fever despite the use of neuraminidase inhibitors, whereas M47 for control is from a patient without prolonged fever. Listed residues are those different from B/Massachusetts/02/2012 that is a strain of Yamagata lineage for the production of vaccine. Highlighted as blue are the residues that are same with B/wis/1/2010 that is a strain of Yamagata linage for vaccine.

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K41-1_BNA-AA    ----------------------------SCPGSTFQKALLISPHRFGETKGNSAPLIIREPFI 120
Hong Kong/45/05  QAVNRSATKGVTLLLPEPEWTYPRLSCPGSTFQKALLISPHRFGETKGNSAPLIIREPFI
Memphis/20/96   QAVNRSATKGVTLLLPEPEWTYPRLSCPGSTFQKALLISPHRFGETKGNSAPLIIREPFI
                                            ***********************************

K41-1_BNA-AA    ACGPKECKHFALTHYAAQPGGYYNGTRGDRNKLRHLISVKLGKIPTVENSIFHMAAWSGS 180
Hong Kong/45/05 ACGPKECKHFALTHYAAQPGGYYNGTRGDRNKLRHLISVKLGKIPTVENSIFHMAAWSGS
Memphis/20/96   ACGPKECKHFALTHYAAQPGGYYNGTREDRNKLRHLISVKLGKIPTVENSIFHMAAWSGS
                ************************** ***********************************

K41-1_BNA-AA    ACHDGKEWTYIGVDGPDNNALLKIKYGEAYTDTYHSYANNILRTQESACNCIGGNCYLMI 240
Hong Kong/45/05 ACHDGKEWTYIGVDGPDNNALLKIKYGEAYTDTYHSYANNILRTQESACNCIGGNCYLMI
Memphis/20/96   ACHDGREWTYIGVDGPDSNALLKIKYGEAYTDTYHSYANNILRTQESACNCIGGDCYLMI
                *****:**********.***********************************************:*****

K41-1_BNA-AA    TDGSASGVSECRFLKIREGRIMKEIFPTGRTQHTEECTCGFASNKTIECACRDNSYTAKR 300
Hong Kong/45/05 TDGSASGVSECRFLKIREGRIIKEIFPTGRIKHTEECTCGFASNKTIECACRDNSYTAKR
Memphis/20/96   TDGSASGISECRFLKIREGRIIKEIFPTGRVEHTEECTCGFASNKTIECACRDNSYTAKR
                *******:************:********  ***********************************

K41-1_BNA-AA    PFVKLNVETDTAEIRLMCTKTYLDTPRPDDGSITGPCESNGGKGSGGIKGGFVHQRMASK 360
Hong Kong/45/05 PFVKLNVETDTAEIRLMCTETYLDTPRPDDGSITGPCESNGDKGSGGIKGGFVHQRMASK
Memphis/20/96   PFVKLNVETDTAEIRLMCTETYLDTPRPDDGSITGPCESNGDKGSGGIKGGFVHQRMASK
                ********************:*********************.*****************

K41-1_BNA-AA    IGRWYSRTMSKTERKGMGLYVKYDGDPWADSDALTFSGVMVSMEEPGWYSFGFEIKDKKC 420
Hong Kong/45/05 IGRWYSRTMSKTERMGMGLYVKYDGEPWADSDALTFSGVMVSMEEPGWYSFGFEIKDKKC
Memphis/20/96   IGRWYSRTMSKTKRMGMGLYVKYDGDPWTDSDALALSGVMVSMEEPGWYSFGFEIKDKKC
                ************:* **********:**:*****: :************************

K41-1_BNA-AA    DVPCIGIEMVHDGGKKTWHSAATAIYCLM----------------
Hong Kong/45/05 DVPCIGIEMVHDGGKETWHSAATAIYCLMGSGQLLWDTVTGVNMAL
Memphis/20/96   DVPCIGIEMVHDGGKKTWHSAATAIYCLMGSGQLLWDTVTGVDMAL
                ***************:*************
```

Fig. 2. Alignment of BNA of oseltamivir-resistant and -sensitive strains
K41-1 is an oseltamivir-resistant strain, whereas others are oseltamivir-sensitive strains of influenza B. Green and yellow shades indicate unique amino acid residues in K41-1 and the sensitive strains, respectively.

```
M2-1_BNA-AA     ------------------------------------LINPHRFGEIKGNSAPLIIREPFI 120
M47             ------------------------------------FQKALLISPHRFGETKGNSAPLIIREPFI
Memphis/20/96   QAVNRSATKGVTLLLPEPEWTYPRLSCPGSTFQKALLISPHRFGETKGNSAPLIIREPFI
Hong Kong/45/05 QAVNRSATKGVTLLLPEPEWTYPRLSCPGSTFQKALLISPHRFGETKGNSAPLIIREPFI
                                                  **.****** ***************

M2-1_BNA-AA     ACGPTECKHFALTHYAAQPGGYYNGTREDRNKLRHLISVKLGKIPTVENSIFHMAAWSGS 180
M47             ACGPKECKHFALTHYAAQPGGYYNGTREDRNKLRHLISVKLGKIPTVENSIFHMAAWSGS
Memphis/20/96   ACGPKECKHFALTHYAAQPGGYYNGTREDRNKLRHLISVKLGKIPTVENSIFHMAAWSGS
Hong Kong/45/05 ACGPKECKHFALTHYAAQPGGYYNGTRGDRNKLRHLISVKLGKIPTVENSIFHMAAWSGS
                ****.********************** ********************************

M2-1_BNA-AA     ACHDGKEWTYIGVDGPDSNALLKIKYGEAYTDTYHSYAKNILRTQESACNCIGDCYLMI 240
M47             ACHDGREWTYIGVDGPDSNALLKIKYGEAYTDTYHSYAKNILRTQESACNCIGDCYLMI
Memphis/20/96   ACHDGREWTYIGVDGPDSNALLKIKYGEAYTDTYHSYANNILRTQESACNCIGDCYLMI
Hong Kong/45/05 ACHDGKEWTYIGVDGPDNNALLKIKYGEAYTDTYHSYANNILRTQESACNCIGGNCYLMI
                *****:**********.*********************:***************:*****

M2-1_BNA-AA     TDGPASGVSECRFLKIREGRIIKEIFPTGRVKHTEECTCGFASNKTIECACRDNRYTAKR 300
M47             TDGPASGISECRFLKIREGRIIKEIFPTGRVKHTEECTCGFASNKTIECACRDNSYTAKR
Memphis/20/96   TDGSASGISECRFLKIREGRIIKEIFPTGRVEHTEECTCGFASNKTIECACRDNSYTAKR
Hong Kong/45/05 TDGSASGVSECRFLKIREGRIIKEIFPTGRIKHTEECTCGFASNKTIECACRDNSYTAKR
                ***.***:**********************:*********************** *****

M2-1_BNA-AA     PFVKLNVETDTAEIRLMCTETYLDTPRPNDGSITGPCESDGDKGSGGIKGGFVHQRMASK 360
M47             PFVKLNVETDTAEIRLMCTKTYLDTPRPNDGSITGPCESDGDEGSGGIKGGFVHQRMASK
Memphis/20/96   PFVKLNVETDTAEIRLMCTETYLDTPRPDDGSITGPCESNGDKGSGGIKGGFVHQRMASK
Hong Kong/45/05 PFVKLNVETDTAEIRLMCTETYLDTPRPDDGSITGPCESNGDKGSGGIKGGFVHQRMASK
                ********************:*********:***********:**:*****************

M2-1_BNA-AA     IGRWYSRTMSKTKRMGMGLYVKYDGDPWTDSEALALSGVMVSMEEPGWYSFGFEIKDKKC 420
M47             IGRWYSRTMSKTKRMGMGLYVKYDGDPWTDSEALALSGVMVSMEEPGWYSFGFEIKDKKC
Memphis/20/96   IGRWYSRTMSKTKRMGMGLYVKYDGDPWTDSDALALSGVMVSMEEPGWYSFGFEIKDKKC
Hong Kong/45/05 IGRWYSRTMSKTERMGMGLYVKYDGEPWADSDALTFSGVMVSMEEPGWYSFGFEIKDKKC
                ************:************:**:**:**: :************************

M2-1_BNA-AA     DVPCIGIEMVHD----------------------------------
M47             DVPCIGIEMVHDGGKTTWHSAATAI----------------------
Memphis/20/96   DVPCIGIEMVHDGGKKTWHSAATAIYCLMGSGQLLWDTVTGVDMAL
Hong Kong/45/05 DVPCIGIEMVHDGGKETWHSAATAIYCLMGSGQLLWDTVTGVNMAL
                ************
```

Fig. 3. Alignment of BNA of zanamivir-resistant and -sensitive strains
M2-1 is a zanamivir-resistant strain, whereas others are zanamivir-sensitive strains of influenza B. Green and yellow shades indicate unique amino acid residues in M2-1 and the sensitive strains, respectively.

(Figs. 2 and 3). For the identification of potential mutations for drug resistance, we compared the sequences of K41-1 and M2-1 to those of drug-sensitive strains. I262M, V271T, K/E272Q, E320K, D342G and M375K are novel mutations found in K41-1. Those are potential mutations for oseltamivir-resistance. S99N, T106I, K125T and S295R are novel mutations found in M2-1, and potential mutations for zanamivir resistance.

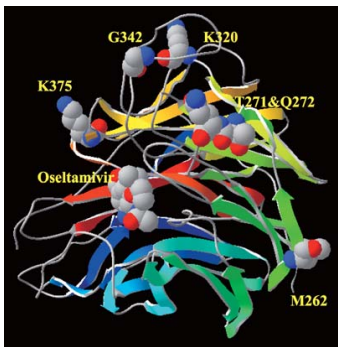

Fig. 4. Mutation sites on K41-1 BNA
Model structure of BNA from K41-1 is depicted as a ribbon model. Oseltamivir molecule and mutated residues compared with oseltamivir-sensitve strains are indicated as spheres.

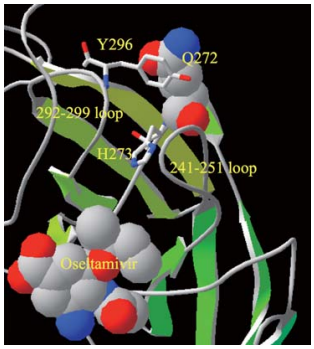

Fig. 5. Enlarged view around Q272 of K41-1 BNA
The backbone structure of the BNA model is depicted as a ribbon model. Q272 and oseltamivir are depicted as spheres. H273 and Y296 are depicted as sticks.

Modeling analysis for drug resistance

Potential sites responsible for oseltamivir resistance were mapped on a structure model of K41-1 BNA (Fig. 4). The 272nd residue is Lys or Glu in the oseltamivir-sensitive strains (Fig. 2). It has been reported that substitutions of H273 and Y296 have impacts on drug resistance[7,8]. Side chains of Q272, Y296 and H273 were arranged like a zipper (Fig. 5). H273, the 241-251 loop and 292-299 loop formed the binding pocket to oseltamivir. The binding pocket was scaffolded by the zipper structure of Q272, Y296 and H273. Thus, mutation at the 272nd residue may perturb the framework structure of the oseltamivir-binding pocket, which may cause the drug-resistance.

The 375th residue is Met in the oseltamivir-sensitive strains (Fig. 2). In the drug-sensitive strains, the side chain of M375 is surrounded by M403 and P406, and contributes to stabilizing the 371-373 loop (Fig. 6). In the modelled K41-1 BNA structure, the side chain of K375 interacted with E373 and was detatched from M403 and P406, which may destabilize the structure of the 371-373 loop. This may cause the destabilization of the interaction between R374 and oseltamivir.

The distances between the oseltamivir-binding site and M262 and K320 are quite long. These residues are located on the surface of BNA, and have no important interaction to other residues. The 271st residue is Val or Ile in the oseltamivir-sensitive strains. Although this residue was replaced with Thr, it was unlikely that this substitution had an impact on the structure of BNA, because the structure of Val/Ile and Thr are quite similar. The 342nd residue is Asp in the oseltamivir-sensitive strains, whereas it was Gly in K41-1. It was unlikely that this substitution had an impact on the structure and function

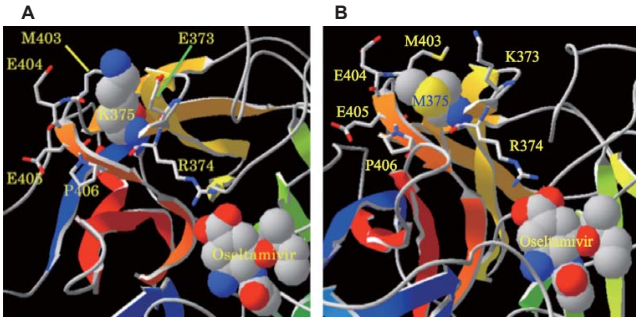

Fig. 6. Enlarged views around K375/M375
A. View around K375 of the K41-1 strain. K375 and oseltamivir are indicated as spheres. K373, R374, M403, E404, E405 and P406 are depicted as sticks. B. View around M375 of oseltamivir-sensitive strain.

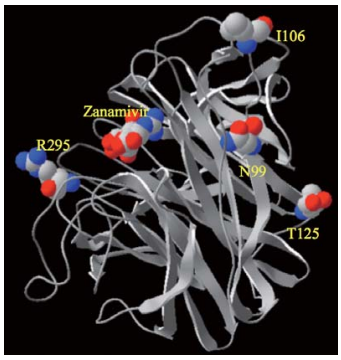

Fig. 7. Mutation sites on BNA as to zanamivir-resistance
The M2-1 BNA model is depicted as a ribbon model. Zanamivir and mutated residues in M2-1 BNA compared with zanamivir-sensitive strains are depicted as spheres.

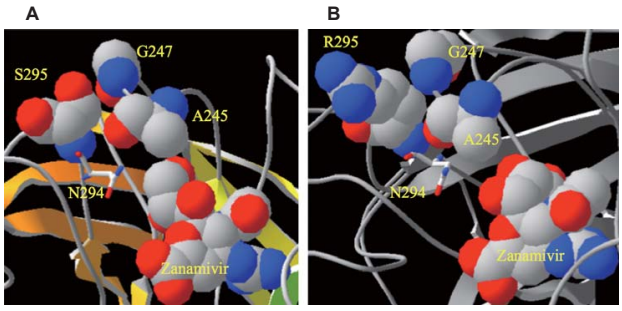

Fig. 8. Enlarged views around S295/R295 of BNA
A View around S295 of zanamivir-sensitive strain, B Corresponding view around R295 of the M2-1 strain.

of BNA, because this residue had no side chain contact to other residues.

Potential sites responsible for zanamivir-resistance, N99, I106, T125, and R295, were mapped on a structure model of M2-1 BNA (Fig. 7). R295 was located next to N294 that composed the framework of the substrate binding pocket (Fig. 8). In zanamivir-sensitive strains, the 295th residue is Ser, which interact with G247 and A245, contributing to stabilization of the structure around N294. Replacing S295 with Arg caused loss of these interactions, which may impair the stability of the binding pocket of BNA. N99, I106, and T125 were far from the zanamivir-binding site, so were unlikely to have impacts on the inhibitor-binding.

Discussion

Clinical observations

According to the past studies, the febrile period of influenza B viral infection was longer than of influenza A viral infection after oseltamivir treatment[2,3]. In the present study, the average febrile period after treatment of neuraminidase inhibitor of influenza B viral infection was significantly longer than influenza A viral infection. The rate of febrile period >48 hours after treatment of influenza B viral infection was also higher than influenza A viral infection. Except for the A/B type, the other characteristics of the subjects including age distribution, vaccination history and secondary bacterial infection did not influence prolonged duration of the fever. Neuraminidase inhibitor-resistant influenza B viruses were rare. The drug-resistant influenza B viruses were isolated from immuno-compromised children with prolonged treatment of neuraminidase inhibitor[9,10]. However, Hatakeyama *et al*. reported that osertamivir- and zanamivir-resistant influenza B viruses were isolated from both ordinary neuraminidase inhibitor-treated and non-treated children in Japan[4]. So far, the nineteen mutations of influenza B viruses related to resistance to neuraminidase inhibitors were reported[7]. However, the precise mechanism and clinical impact of resistance of influenza B viruses to neuraminidase inhibitors were not clarified.

Structure change of neuraminidase gene of influenza type B isolated at clinics in Chiba Prefecture: Drug resistance analyzed in modeling of the neuraminidase with novel mutations

We performed extensive sequence analysis of BNAs of both oseltamivir-resistant and zanamivir-resistant samples with prolonged fever. The analyzed sequences indicated numerous substitutions in comparison with BNA of the Yamagata type for the production of vaccine. In addition, the sequences showed novel mutations in both oseltamivir-resistant and zanamivir-resistant BNAs. We built structural models of mutant BNAs to analyze impacts of the mutations on drug resistance, and found that two mutations of the oseltamivir-resistant strain, K/E272Q and M375K, and one mutation of the zanamivir-resistant strain, S295R, may be responsible for drug resistance. All these mutations were located on the framework of the substrate-binding pocket of BNA, but lacked direct interaction with the bound drugs. The analysis suggested possibilities that these mutations may destabilize the structure of the framework, which may lead to weak binding of BNA to the drugs.

E119V, R292K, H274Y and N294S of neuraminidase of influenza A (ANA) were reported previously as drug resistance mutations. E119, R292, H274 and N294 of ANA correspond to E117, R292, H273 and N294 of BNA. E119 and R292 are direct binding residues to the drugs including oseltamivir and zanamivir. In the mutated protein with either E119V or R292K, direct binding between ANA and the drugs were predicted to be lost, leading to loss of inhibition by the drugs[11]. In contrast, H274 and N294 are framework residues, which indicate that mutations in the framework can cause drug resistance. Several possible mechanisms for the drug resistance with the H274Y and N294S mutations have been proposed based on simulation studies for ANA. According to these studies, it may be difficult to generalize the resistance mechanisms of H274Y and N294S.

The complex structure of ANA with H274Y-oseltamivir (PDB code: 3CL0) revealed that the bulkier side chain of Y274 presses out that of E276 towards the substrate-binding pocket in relative to WT[12]. Thus, it was proposed that such a structural

change hampers the interaction with oseltamivir. A computational simulation study proposed that infiltration of water molecules into the substrate-binding pocket causes the drug resistance of the H274Y mutant[13]. The other group proposed that the mutation on either H274 or N294 impairs binding to the drugs because H274 and N294 are located on the charged binding funnel[14].

Another study based on simulation indicated that the mutations of H274Y and N294S shifted the locations of E276, E277 and R292 and proposed that such structural changes have impacts on interaction with oseltamivir and zanamivir[15]. E277 and R292 are the residues that directly interact with the drugs, whereas E276 is one of the framework residues. In the simulation of the N294S mutant by Ripoll *et al*., a hydrogen bond between S294 and E276 was formed and the main chain carbonyl of Y347 was flipped. As a consequence, Y347 interacted with the main chain carbonyl of R292. These had an impact on the dynamics of R292 that directly bound to zanamivir and oseltamivir, which may cause drug-resistance. The other simulation study proposed that the hydrogen bonds between R118 and the drugs are weakened in the N294S mutant[11].

As discussed above, several different hypotheses for the mechanism of drug-resistance by the framework mutations including H274Y and N294S of ANA have been proposed thus far. However, it is commonly presumed that the mutations have impacts on the subtle internal structure and dynamics of ANA, which cause the resistance. Thus, it is likely that the framework mutations reported here including K/E272Q, M375K and S295R may have impacts on the structure and/or dynamics of the framework.

This study has a limitation. In this study, we determined the mutation sites and the suggestive resistant mechanism from the analysis of molecular modeling of neuraminidase structure from influenza B virus RNA obtained from patient. However, to clarify the relationship to the mutation to the neuraminidase inhibitors resistance more precisely, further studies such as the determination of the rise of IC_{50} level against neuraminidase inhibitors using the virus possessing the mutations are required. Therefore, in the future study, it is better to isolate not only the RNA but also the virus from the patient sample for the analysis of IC_{50} level against neuraminidase inhibitors.

Acknowledgments

This work was supported in part by grants-in-aid for Scientific Research by the e-ASIA Joint Research Program from Japan Agency for Medical Research and Development, AMED, Japan.

Disclosure

The authors have no financial conflict of interest.

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ADC LABORATORIES



【ADC Members】

Director: Kazuo Suzuki, Vice-Director: Shoji Kawachi

Specially Appointed Professor: Tomoko Yamamoto

Associate Professor: Shoichi Suzuki, Assistant Professor: Ryuichi Sugamata

Research Assistant: Fuyu Ito, Secretary: Kaoru Tosaka, Haruko Haisa

Graduate student: Thuy Thu Nguyen (D3), Tran Huu Dat (D2)

【Research Presentations: The 22nd MPO Meeting】

ADC staff presented our research at the 22nd MPO meeting in Kyoto University. from Dec. 2 to 3.

■ Moesin expression level of MPO-ANCA associated vasculitis mouse
Ito F. *et al.*

■ The inhibitory activity of macrolide derivatives in proliferation of 2009 pandemic influenza A/H1N1 viruses (H1N1pdm09)
Dat T.H. and Sugamata R. *et al.*

■ Effects of hypothiocyanite on airway epithelial cells
Nguyen T.T. and Suzuki S. *et al.*

■ Analysis of 21 cases of influenza-associated severe acute respiratory distress syndrome in Vietnamese children
Kawachi S. and Suzuki K. *et al.*


第22回MPO研究会開催概要

2016年12月2日（金曜） 京都大学 楽友会館 2階会議室

14:00-14:05 開催挨拶
14:05-16:45 一般演題
16:45-17:00 休憩
17:00-18:00 特別講演
京都大学医学部附属病院 免疫・膠原病内科 講師 吉藤元先生
「大型血管炎の基礎と臨床」

19：30頃～懇親会 （希望者のみ）

2016年12月3日（土曜） 芝蘭会館別館（国際交流会館）研修室

9:00-11:50 一般演題
11:50-12:00 閉会挨拶・次回開催案内

当番世話人
京都大学医学部附属病院 臨床研究総合センター 猪原 登志子
(公財) 田附興風会医学研究所北野病院腎泌尿器センター腎臓内科 武曾 恵理
(公財) ルイ・パストゥール医学研究センター 宇野 賀津子


Attendee of MPO Meeting in Kyoto

INTERNATIONAL MEETING AND SYMPOSIUM

開催したイベント （2016.7.1～2016.12.31）

日程	イベント名	演者など	
2016年10月17日（月）～10月18日（火）	e-ASIA Project Meeting	Annual Meeting	Manila, Philippines
2016年9月21日（水）	危機管理と防災	防災訓練	大学棟
2016年9月16日（金）16:00～18:00	ADC 50th Anniversary Symposium	臨床大講堂	
2016年8月26日（金）13:00～18:00	TAVP 報告会	医学部5年生 7名	本部棟 2F
2016年8月14日（日）～8月20日（土）	TAVP Training for 7 Students (5-year)	NCH and HMU	Hanoi, Vietnam
2016年7月5日（火）	内閣官房、厚労省の訪問	ADC Members	ADC Lab

今後のイベント情報 （2017.1.1～2017.6.30）

日程	イベント名	演者など	
2017年2月	第13回国際シンポジウム	大学棟104講義室	
2017年2月	バイオセーフティ講習会	ADC	セミナー室