2nd Conference on International Asian Pediatric Infectious Diseases

Date: 2015 June 20th (Sat) Venue: The University of Tokyo, No. 2 Medical Building, Small Hall Access Map: http://www.u-tokyo.ac.jp/campusmap/cam01 02 03 j.html

Program

10:00-10:05	Dr. Hiroshi Ushijima: Opening remarks
10:05-10:10	Dr. Masashi Mizuguchi: Welcome remarks

*Discussion time: 5 min after each presentation

10:00-12:15	Session	1
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Chair: Dr. Yuki Eshita	
10:10-10:40	Dr. Yuki Eshita: Susceptibility of Japanese Aedes (Stegomyia) mosquitoes to arboviruses
10:45-11:15	Dr. Kenji Abe: Rubella outbreak among children in Vietnam: Molecular characterization of virus and its pathogenesis in human fetuses
11:20-11:50	Dr. Hideo Negishi: Regulation of pattern recognition receptor signaling -Control of gene induction by IRF family transcription factor-
11:55-12:10	Dr. Tsuguto Fujimoto: A surveillance of adenoviruses in patients with respiratory and ocular Infections

12:15-13:30 Lunch Time

13:30-15:45 Session 2 Chair: Dr. Tsuguto Fujimoto

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13:30-14:00	Dr. Hideki Kohno: Recent advances of RNA virus measurement with real-time PCR and development of anti-viral agents feline calicivirus
14:05-14:20	Dr. Masashi Mizuguchi: CNS complications of pediatric infectious diseases
14:25-14:40	Dr. Hiroshi Ushijima: Overview of our recent research on infection in children

14:45-15:15	Dr. Tuan A. Nguyen: • Rotavirus diarrhea in the children's hospital 1, Ho Chi Minh city. • Clinical manifestations and management of peptic ulcer diseases in children at No.1 Children's hospital from June 2013 to January 2014. • Topics of infection in children in Asia and Vietnam (No abstract)
15:20-15:40	Dr. Shoko Okitsu: Cosavirus in Humans and Pigs

15:45-16:00 Coffee Time (Flexible)

16:00-17:40 Session 3

Chair: Dr. Kenji Abe

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16:00-16:15	Dr. Tomoyuki Shiota: Searching for the host factors involved in hepatitis E virus infection
16:20-16:35	Dr. Pattara Khamrin: Molecular characterization and zoonotic potential analysis of rotaviruses detected in children and piglets with diarrhea
16:40-16:55	Dr. Aksara Thongprachum: Structural analysis of histo-blood group antigen binding specificity of norovirus variants
17:00-17:30	Dr. Sayaka Takanashi: Rotavirus infection in the transitional period of rotavirus vaccine implementation
17:35-17:40	Dr. Hiroshi Ushijima: Closing remarks

Evening Party: 18:00-20:00 (2hrs) Venue: **Chimney** チムニー 本郷店 TEL050-5789-3083 http://r.gnavi.co.jp/g213900/photo/ Charge (tentative): Students-free of charge, Others-3500Yen

Susceptibility of Japanese Aedes (Stegomyia) mosquitoes to arboviruses

Yuki ESHITA

Department of Infectious Disease Control, Faculty of Medicine, Oita University, Oita, Japan,

Since about 200,000 typical clinical cases of dengue patients were recorded in Nagasaki, 1942, we had no autochthonous dengue patients except imported cases in Japan. In August, 2014, however dengue epidemic occurred suddenly at Yoyogi Park in Tokyo, Japan. About 160 dengue patients were reported totally. Dengue is an infectious diseases caused by dengue viruses include serotype 1 to 4 and vector mosquitoes. Vector control is one of the most effective strategies for arboviral diseases since no vaccine available. Since imported cases of arboviruses including dengue, chikungunya and Rossriver patients have been reported in Japan, in this text, we summarized the susceptibility of Japanese Aedes mosquitoes to dengue virus by using RT-PCR and RT-LAMP methods. Dengue virus positive results were obtained by oral infection in the following 5 Aedes (Stegomyia) species, Ae. albopictus, Ae. flavopictus flavopictus, Ae. f. miyarai, Ae. riversi, and Ae. galloisi in Japan. And also, chikungunya and Ross River virus positive results were obtained the following 3 Aedes species, Ae. albopictus, Ae. f. flavopictus, and Ae. riversi. All these results reveal that Aedes albopictus is suspected epidemiologically and ecologically as one of the most important arboviral vector in Japan.

This study is the collaboration with Drs. Masako Fukuda, Lucky R. Runtuwene, Kaori Noguchi, Shinya Hidano, Naganori Kamiyama, Kyoko Hayashida, Raweewan Srisawat, Narumon Komalamisra, Hironari Narita, Hiroshi Ushijima, Arthur E. Mongan, Josef Tuda, Mihoko Imada, Junya Yamagishi, Chihiro Sugimoto, Ryuichiro Maeda, Yutaka Suzuki, Tomohiko Takasaki, Ichiro Kurane, and Takashi Kobayashi.

Rubella outbreak among children in Vietnam: Molecular characterization of virus and its pathogenesis in human fetuses

Kenji ABE

Department of Pathology, National Institute of Infectious Diseases, Tokyo, Japan

Aim:

Rubella remains poorly controlled in Southeast Asia, including Vietnam. The aim of this study was to carry out molecular-based characterization and genotyping of the rubella virus prevalent in Vietnam during the 2011-2012 period. In addition, development of congenital rubella syndrome (CRS) associated with rubella virus infection during pregnancy is clinically important, but its pathogenicity of the virus is still unclear.

Patients and Methods:

Amniotic fluid and throat swab samples were collected from 130 patients (110 cases from pregnant women and 20 cases from fetuses/newborns). Viral RNA was obtained directly from clinical specimens or cultured cells inoculated with virus in 4 cases, amplified by PCR, and then the E1 gene containing 739 nucleotides recommended by the WHO to identify the viral genotypes was sequenced.

Furthermore, pathological examination in 3 aborted fetuses with congenital rubella infection was also conducted.

Results:

By screening with real-time PCR, viral RNA was detectable in amniotic fluids from 103 out of 110 (93.6%) pregnant women and in the throat swabs from all of 20 (100%) fetuses/newborns. In addition, viral RNA was also detected in the placenta from all cases of fetuses/newborns. All of 20 fetuses/newborns presented with congenital cataract. Twenty-four isolates with the E1 gene were obtained by nested RT-PCR and then sequenced. Using phylogenetic analysis with rubella reference sequences, all of the isolates were found to be genotype 2B. Interestingly, 94% (31/33) of Vietnamese isolates, including 9 strains from the database, formed an independent cluster within the genotype 2B suggesting that indigenous viruses are prevalent in this region.

At autopsy, all 3 aborted fetuses showed congenital cataract confirmed by gross observation. Rubella virus was infected through systemic organs including circulating hematopoietic stem cells, but major histopathogical changes were found in the liver. It is noteworthy that the virus was infected in the ciliary body of the eye. **Interpretation**:

Our results indicate that rubella virus spread in Vietnam during the 2011-2012 belonged to the genotype 2B. Importantly, our study based on pathological examination demonstrated that rubella virus was infected through systemic organs in human fetuses. This fact has been confirmed by immunohistochemistry and direct detection of viral RNA in multiple organs. To the best of our knowledge, this study is the first report proved that the virus is infected in the ciliary body suggesting the possible causes of cataracts. Information about genetic characterization of RV prevalent in Vietnam has improved, which should aid in the control of rubella and CRS in this region.

Collaborators:

Van H. Pham (School of Medicine, University of Medicine and Pharmacy in Ho Chi Minh City), Thong V. Nguyen (Hung Vuong Hospital), Diem P.H. Nguyet, Khanh N.H. Mai and Khanh H. Truong (Children Hospital No.1) in Ho Chi Minh City, Vietnam.

This study has been reported in:

- Van Hung Pham, Thong Van Nguyen, Truc Thanh Thi Nguyen, Linh Duy Dang, Ngoc Hieu Hoang, Truong Van Nguyen, Kenji Abe: Rubella epidemic in Vietnam: Characteristic of rubella virus genes from pregnant women and their fetuses/newborns with congenital rubella syndrome. *Journal of Clinical Virology* 57: 152-156, 2013 (DOI: 10.1016/j.jcv.2013.02.008)
- 2. Thong Van Nguyen, Van Hung Pham, Kenji Abe: Pathogenesis of congenital rubella virus infection in human fetuses: Viral infection in the ciliary body could play an important role in cataractogenesis. *EBioMedicine* 2: 59-63, 2015 (DOI:10.1016/j.ebiom.2014.10.021)

ベトナム・ホーチミン市における風疹と麻疹の流行とその特徴

国立感染症研究所 感染病理部、ベトナム・ホーチミン医科薬科大学

阿部賢治

目的:東南アジア地域で今臨床的に問題となっている感染症の実態とその特徴を解明する ことを目的とする。今回は、ベトナム・ホーチミン市で近年大流行した風疹と麻疹の特徴 を解明した。

患者と方法:

1)風疹:2011~2012年にかけてベトナム・ホーチミン市で集団発生し、臨床的に風疹が 疑われた患者 130例(妊婦 110例、新生児・胎児 20例)を対象とした。妊婦の羊水と新生 児/胎児の咽喉ぬぐい液や胎盤を採取し、風疹ウイルス RNA を PCR 法にて検出した。PCR 陽性を示した 24例を対象に、E1遺伝子の塩基配列を決定し、分子系統樹解析を行い、ゲ ノタイプを同定した。また、中絶胎児 3例の剖検を実施し、その病理学的所見を観察した。 2)麻疹:2014年にベトナム・ホーチミン市で集団発生し、臨床的に麻疹が疑われた 122 例の小児患者を対象とした。全例で咽喉ぬぐい液と 31 例から PBMC を採取し、麻疹ウイ ルス RNA を PCR 法にて検出した。更に、N と H 遺伝子の塩基配列を決定し、分子系統樹 解析を行い、ゲノタイプを同定した。

結果:

1)風疹の分子疫学: PCR 法にて風疹ウイルス RNA が妊婦の羊水中には 110 例中 103 例 (93.6%)、新生児・胎児の咽喉ぬぐい液には 20 例中 20 例で陽性を示した。更に、新生 児・胎児の胎盤からも全例でウイルス RNA が検出された。新生児・胎児では全例で白内 障が観察された。PCR 陽性を示した 24 例を対象に、E1 遺伝子の塩基配列を決定し、分子 系統樹解析からゲノタイプを同定した結果、全例でゲノタイプ 2B に属した。興味あるこ とに、今回分離されたゲノタイプ 2B ベトナム株は他の 2B 株から離れた位置に独立したク ラスターを形成したことから、ベトナム国内では独自の土着風疹ウイルスが長い間流行を 繰り返していることが推察された。また、日本国内で 2011 年に分離された一部の風疹ウイ ルス株がベトナム株と同一のクラスターに属したことから、ベトナム由来のウイルスが国 内に入ってきていることが推察された。

2)風疹の感染病理: 剖検を行った中絶胎児3例は、肉眼的にいずれも白内障と肝脾腫が 観察された。PCR 法および免疫染色法で造血幹細胞を含む全身臓器に風疹ウイルスの感染 が確認された。病理組織所見では、特に肝臓において病変が顕著であった。特筆すべきは、 免疫染色で眼球の毛様体細胞にウイルスの感染が確認できたことである。この所見は初の 報告であり、白内障の成因に関与している可能性が示唆された。

3) 麻疹の分子疫学:122 例全例の咽喉ぬぐい液から、麻疹ウイルス RNA が検出された。 また 31 例全例の PBMC からもウイルス RNA (マイナス鎖とプラス鎖両方) が検出された。 118 例から分離された N 遺伝子の塩基配列を決定し、分子系統樹解析からゲノタイプを同 定した結果、全例がゲノタイプ D8 に属した。興味あることに、今回分離されたゲノタイ プ D8 ベトナム株は他の D8 株から離れた位置に独立したクラスターを形成した。この独自 のクラスターの分岐部は、99%のブートストラップ値(絶対値)を示した。また他の D8 レファランス株と比し 3.3% の塩基配列相違を示した。しかし、H 遺伝子領域では、他の D8 レファランス株から 1.1% と低い塩基配列相違であった。興味あることに、今回分離さ れた D8 ベトナム株全てで、N 遺伝子領域においてユニークなアミノ酸配列(R442, S451, G452)が観察された。

考察:

東南アジア地域における風疹と麻疹の疫学実態は未だ不明な点が多い。ベトナム・ホー チミン市で近年大流行したこの2種の感染症は、母子保健上重要であり、行政上早急な対 策が望まれる。今回我々が報告したこの地域に流行する風疹と麻疹ウイルスの特徴解明は、 WHOが進めるワクチンによるこの感染症制圧に寄与することを願っている。

また風疹の感染病理学的特徴に関する報告は極めて少なく、このため小児の感染症で重要な位置を占める先天性風疹症候群(CRS)の成因は未だ不明のままである。今回我々が報告した風疹胎児における病理所見は、CRSの病態とその成因を解明する上で大変重要である。特に、毛様体における風疹ウイルス感染の証拠が得られたのは初の報告であり、先天性白内障の成因として重要な役割を担っている可能性が示唆された。

本研究は、Van H. Pham (ホーチミン医科薬科大学医学部分子バイオメディスン)、 Thong V. Nguyen (フン・ブン産科病院病理部)、Diem P.H. Nguyet, Khanh N.H. Mai, Khanh H. Truong (ベトナム・小児第一病院呼吸器科・感染症科)との共同研究である。

発表論文

- Van Hung Pham, Thong Van Nguyen, Truc Thanh Thi Nguyen, Linh Duy Dang, Ngoc Hieu Hoang, Truong Van Nguyen, Kenji Abe: Rubella epidemic in Vietnam: Characteristic of rubella virus genes from pregnant women and their fetuses/newborns with congenital rubella syndrome. *Journal of Clinical Virology* 57: 152-156, 2013 (doi: 10.1016/j.jcv.2013.02.008)
- Van H. Pham, Diem P.H. Nguyet, Khanh N.H. Mai, Khanh H. Truong, Loc V. Huynh, Trang H.T. Pham, Kenji Abe: Measles epidemics among children in Vietnam: Genomic characterization of virus responsible for measles outbreak in Ho Chi Minh City, 2014. *EBioMedicine* 1: 133-140, 2014 (DOI:10.1016/j.ebiom.2014.10.015)
- 3. Thong Van Nguyen, Van Hung Pham, Kenji Abe: Pathogenesis of congenital rubella virus infection in human fetuses: Viral infection in the ciliary body could play an important role in cataractogenesis. *EBioMedicine* 2: 59-63, 2015 (DOI:10.1016/j.ebiom.2014.10.021)

Regulation of pattern recognition receptor signaling -Control of gene induction by IRF family transcription factor-

Hideo NEGISHI

Department of Molecular Immunology, Institute of Industrial Science, The University of Tokyo

Pathogens are recognized as a non-self and attacked by immune system. The first step of pathogen recognition is mediated by pattern recognition receptors (PRRs) which are expressed in many types of cells including innate immune cells such as dendritic cells, macrophages and so on. While adaptive immune system recognizes specific molecular structure of the pathogens, PRRs recognize conserved molecular patterns (pathogen associated molecular patterns: PAMPs) with relatively low specificity. In response to the recognition of PAMPs by PRRs down stream signaling is activated and a variety of cytokines such as type I IFN and pro-inflammatory cytokines are produced, resulting in the immediate activation of immune response. Although PRRs are firstly identified as a pathogen recognition receptor, recent study revealed that PRRs also recognize the endogenous component released from dead cells, leading to the exacerbation of autoimmune and inflammatory diseases.

I started my study on PRRs in 2002 at department of immunology in Tokyo University (Tadatsugu Taniguchi's lab). In those days, they were mainly studying on the regulatory mechanism of pathogen (virus in particular)-derived type I gene induction; however, more upstream event involved in the pathogen recognition or signal transduction remains unclear. The discovery of PRRs enabled us to study IRF-mediated gene induction in the context of signaling pathway activated in down stream of PRRs. Thereafter, I have been studying on the molecular regulation mechanism and physiological role of PRR signaling through the analysis of IRF family transcription factors. In this presentation, I will introduce my study on PRR signaling with recent results.

免疫応答を惹起するパターン認識受容体シグナルの制御機構 -IRFファミリー転写因子を中心とした遺伝子発現制御—

東京大学 生産技術研究所 炎症・免疫制御学社会連携研究部門 根岸英雄

生体は病原体を異物として認識し、免疫応答を活性化します。そのはじめの病原体認 識に関わる受容体がパターン認識受容体(pattern recognition receptor :PRR)であり、マクロ ファージや樹状細胞などの自然免疫系細胞をはじめとし、様々な細胞に発現していること が分かっています。適応免疫系が分子構造を高い特異性で認識するのに対し、PRR は比較 的低い特異性で分子パターンを認識し、一つの受容体に対して複数のリガンドが存在する こともあります。PRR に病原体成分が認識されると、細胞内の下流シグナルが活性化し、I 型 IFN や様々なサイトカインの遺伝子発現誘導等を介して、迅速に免疫応答が活性化され ます。PRR は病原体認識受容体として発見されましたが、その後、生体の内在性分子を認 識することも判明し、自己免疫疾患や炎症性疾患の増悪に関わることも分かっています。 すなわち、PRR 経路の活性化は様々な免疫応答の「起点」であり、その制御は多くの免疫 関連疾患の治療や予防に結びつく可能性があります。

私は 2002 年に東京大学の免疫学教室 (谷口維紹教授)にて PRR の研究を開始しました。 当時の免疫学教室では、病原体(特にウイルス)の感染によって誘導される I 型 IFN 遺伝子 の発現制御機構について、IRF ファミリー転写因子を中心に研究がなされていました。し かしながら、転写レベルの制御機構が解明される一方で、病原体の認識に関わる受容体や 転写因子の活性化に至るまでのシグナル経路について多くの部分が不明なままでした。

そのような中、Toll 様受容体(TLR)の発見によって病原体成分を認識する受容体が同定 され、その後、その下流のシグナル分子が次々に同定されました。これらの発見によって IRF ファミリー転写因子による遺伝子発現制御機構を明確な位置づけで、シグナル伝達経 路の流れの中で研究できるようになりました。その後さらに、ウイルス由来の RNA を認 識する RIG-I の同定をはじめ、様々な PRR 受容体が相次いで発見され、この分野は大きく 発展しました。私はそのような様々な受容体の下流で引き起こされる現象がどのように制 御され、また、生理的な重要性を持つかについて、遺伝子発現調節の観点から、特に IRF ファミリー転写因子を通じて研究を進めてきました。

本発表では遺伝子発現制御機構の解析を通じて明らかにした PRR 応答の制御機構や生 理的な重要性、疾患との関わりについて、特に IRF ファミリー転写因子の研究を中心に最 近の知見も含めてご紹介させて頂きます。

A surveillance of adenoviruses in patients with respiratory and ocular infections

Tsuguto FUJIMOTO

Infectious Disease Surveillance Center, National Institute of Infectious Diseases

Purpose: Human mastadenovirus (HAdV) is one of the most common cause of acute respiratory of children and ocular infection of adults. To identify the types of HAdVs causing respiratory and ocular infection in Shizuoka prefecture, a sentinel surveillance was conducted in 2011-2015, including new genotypes (type 52 and above).

Methods: HAdVs were detected and identified by multiplex PCR and PCR sequencing for respiratory samples. Ocular HAdVs were detected and identified by multiplex PCR, PCR sequencing, and specific PCR for new genotypes. Hexon and fiber coding regions were used to identify 256 respiratory and 85 ocular HAdVs.

Results: Through this study, respiratory HAdVs (n =256) were detected and typed as 1 (n = 44), 2 (n = 90), 3 (n = 89), 4 (n = 10), 5 (n = 16), 8 (n = 1), 56 (n = 1), dual detection of 1& 2 (n = 1), and 1& 4 (n = 1). Ocular HAdVs (n = 85) were detected and typed as 2 (n = 1), 3 (n = 26), 4 (n = 7), 5 (n = 1), 8 (n = 1), 11 (n = 2), 37 (n = 27), 53 (n = 4), 54 (n = 3), 56 (n = 10), 64 (n = 1), 48 related recombinant strain (n = 1), and dual detection of 1&3 (n = 1).

Discussion: HAdV circulate as a causative agent of respiratory and ocular infections. HAdV-D56 was transmitted from EKC parents to their child and caused pharyngitis, conjunctivitis and fever (38.5C). This case suggested that HAdV-56 cause EKC in adults and pharungoconjunctival fever (PCF) in children. HAdV-48 related recombinant form [Fujimoto et al. 2015] was isolated again from a patient with conjunctivitis which is the second detection after first detection in Japan 2012. These findings emphasize the importance of the prevention of the family transmission of HAdVs which sometimes fatal for children and immunocompromised.

CONCLUSIONS: This study confirmed that at least 13 kind types of HAdV were cocirculating and caused respiratory tract and ocular infections and some types caused both kinds of infections. These finding underscore the importance of the public sanitation measures against HAdV infections which are sometimes fatal.

Recent advances of RNA virus measurement with real-time PCR and development of anti-viral agents feline Calicivurus

-What our laboratories worked to measure active virus measurement by use of with real time PCR and successful development of anti-viral agents in peroxides-

Hideki KOHNO

*College of Industrial Technology, Nihon University and Hoshi University

Our research was based on the technology how to precise measurement of real, active virus in speedy way to utilize the data instantly to therapy and most efficient way for the benefit of the patient for timely therapy. These concept was highly evaluated as simple and sensitive active virus counting.

In this sense, one of the most practical techniques for virus research was shown in this paper. At first stage, our studies came from rapid and simple clinical investigation and one of the typical work was introduced gene related screening for infectious common flu-virus influenza. This work faced to determine and discriminate influenza A or B type, because we select to medicine from the result on the laboratory testing.

In this way, our understanding was to survey the relationship between virus particles and infectivity. Our observation came from the fact that the real- time PCR reading cycle and numbers of virus particles were closely related.

This means that the more virus particles, the shorter recycling period.

It was so clear relationship, this means even for the virus like Norovirus, the infectivity was easily calculated and co-related to virus numbers and infectivity.

The real data was tested to use feline Calicivirus which is easily cultivated on the cell line. Based on the fact, our study to search the Norovirus infectivity with the use of real time PCR. These methods lead to the development of the most efficient anti-virus agent to select the chemical and furthermore the potent peroxides of benzoic acid derivatives. In the process of screening the chemicals, these agents were closely related to chain length of organic alkyl benzoic acid in peroxide forms.

In the step we are successful to develop novel type of anti-virus agent to evaluate in our testing method.

In this session, author likes to present how to measure the virus particles and RNA in active form, and the unprecedented anti-virus agent in practical ways.

This study is the collaboration with Drs. Tomoe Komoriya, Kazuaki Yoshimune, Marika Hoshi, Kinue Iizuka, Seiici Tobe, Takenori Shimizu, and Masahiro Ogawa.

日大生産工学部におけるウイルス粒子測定と感染価の迅速的測定研究

日本大学生産工学部、日本大学医学部、星薬科大学神野英毅

日大生産工学部では多くのウイルスの測定、臨床検査学的研究をおこなっているが 今回 小児感染症の発表にあたって、感染価と示す active type のウイルスを real-time RT-PCR を用いて定量測定し、ウイルスの定量分析法を提示、測定結果を RNA virus 量と 感染価として細胞を用いた感染価実験と比較検討した。

結果は良く相関をしていたが、さらに Data の正確さを求めるため、感染細胞を pronase や RNAase により分解することにより正確に数値レベルで測定が可能であることが示唆された。さらに 本結果から培養困難なウイルス群、例えばノロウイルスに等に適用できることが解り、臨床レベルでこれらの感染性を示すウイルスが real-time RT-PCR を行うことにより正確に測定できることが解った。

我々はさらに、この結果を基として、カリシウイルスの除菌剤の研究開発に進み、従来の 次亜塩素酸と比較して生体的に日常使いやすい、安息香酸誘導体を過酸化炭酸

(percarbonate)処理により過酸化物である acyloxybenezene(OBS)や acyloxybenzoic acd(OBC)に誘導し、さらに過安息香酸のアルキル鎖を伸ばすことにより除菌性の効果的な 化合物を合成できた。また アルキル基の鎖長の比較をし、ウイルスにもっとも親和性の 良いとされる C12 過安息香酸が最も除菌剤として優れていることが分かり、本剤の使用 により、ウイルスの感染を効果的に防御することを見出した。

これらの除菌剤の研究を可能としたことは、従来よりも簡便迅速に感染価のあるウイルス を測定することに成功した技術研究によるものである¹⁾。

1) S.Tobe et.al J.Oleo Sci. 61,(4) 211-216(2012)

本研究は戸部 聖一、小森谷友絵、吉宗一晃、星痲利華、飯塚 絹江、清水武則、小川眞 宏との共同研究である。

CNS complications of pediatric infectious diseases

Masashi MIZUGUCHI

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Our department has traditionally been devoted to studies of pediatric infectious diseases. Since 2007, researches on the development of child's brain have gained momentum. At present, infection and neuroscience are two main fields of our study. In this meeting, I will summarize our recent achievements on acute encephalopathy following viral and bacterial infections.

小児期感染症の中枢神経系合併症

東京大学大学院医学系研究科発達医科学

水口 雅

発達医科学教室では従来から小児の感染症の研究が盛んであった。2007年以降は神経・発達に関する研究が増え、現在では感染と神経が研究の2本柱である。ここでは小児の感染症の中枢神経合併症、とくにウイルス・細菌感染に続発する急性脳症に関する当教室の最近の成果を述べる。

Overview of our recent research on infection in children

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Infectious diseases and its prevention are important children health issues. We have conducted the studies mainly on diagnosis, molecular epidemiology, pathology, treatment and prevention of viral gastroenteritis for long time. Recently, the expansions of the studies of respiratory and central nervous infections have been set up in our laboratory. The target pathogens are not only viruses but also bacteria. The research setting areas are in Japan and other Asian countries. At this moment, children in Thailand, Vietnam, Indonesia and Bangladesh are mainly included in our study. Animal viruses are also included for the zoonosis research.

Multiplex (RT-) PCR and immunochromatography have been developed for the detections of several microbes. The evaluation among the methods and detection kits were also conducted. Relation between blood group antigen and norovirus has been studied. The titers of antigens, antibodies and copy numbers of norovirus and rotavirus are examined during the course of the diseases. We are also working on the development of rotavirus and norovirus vaccines. For the vaccine project, VLPs have been produced and used in animal (mice) experiments. Evaluation of vaccines and the effect to the environment are also in our research fields. These researches have been proceeded with our research members including physicians.

私たちの最近の小児感染症の研究

日本大学医学部病態病理学系微生物学分野 牛島廣治

小児においては感染症およびその予防が大きな課題である。私たちはウイルス性下痢 症を中心に診断、分子疫学、病態、治療、予防の研究を行ってきた。現在では、一部、呼 吸器感染、中枢神経系そしてウイルスのみならず細菌感染症についても研究を行っている。

研究の対象は日本およびアジア(タイ、ベトナム、インドネシア、バングラデシュ) の小児であるが、一部は大人、そして人獣共通感染症として動物(ブタなど)も行っている。

診断においては Multiplex PCR の対象の微生物をより充実させる。さらにイムノクロ マトの対象を増やすこととともに、診断薬の感度・精度を評価する。病態については特に ノロウイルスについて組織血液型抗原や、感染経過中の抗原抗体の動向を見る。ロタウイ ルス、ノロウイルスのワクチンをわが国での開発を視野に入れての動物実験を行っている。 またワクチンの評価ならびに環境中への影響を含めて今後とも検討する。

Rotavirus diarrhea in the children's hospital 1, Ho Chi Minh City

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Objective: To determine the epideminological and clinical features as well as VP4 and VP7 coding gene among rotavirus acute diarrhea patients.

Methods: Prosspective description and case series in children, who ranged from 2 months to 5 years with acute diarrhea admitted in Dept of Gastroenterology, Children's Hospital No 1.

Results: A total of 316 childrens who ranged in age 2 months- 5 years with acute diarrhea were enrolled in the trials. A total of 173 fecal samples were positive for rotavirus group A antigen giving an overall prevalence of 54.8% (173/316). Mean age of rotavirus diarrhea was 14.5 months (11.2-19 months). More than 88% (153/173) of all diarrheal rotavirus related case were indentified among children aged less than 24 months, with a peak of incidence infection between 12- 24 months 48.6% 984/173). More than 78% of all diarrheal related rotavirus are classified as servere according to the Vesikari score (score \geq 11 points). A total of 90 positive rotavirus specimens were examined for G and Pgenotype. Genotype P[8] was prominent (61.1%), followed by P[4] (37.8%). P[9] was first reported in Vietnam in this study (1.1%). Genotype G consits of G1 (50%), G2 (31.1%), and G3 (18.9%). The G1P[8] strains was indentified as the most prevalent (43.3%) followed by G2P[8] (31.1%), G3P[8] (17.8%), and G3P[9] (1.1%). VP4 genes of P[9] indentified in this study were most closely related to animal, suggesting an animal origin of this rotavirus strain.

Conclusions: Rotavirus acute diarrhea is prevalent among in hospitalized diarrhea childrens. The common worldwide serotypes and G and P combinations co- circulate in this study. This report is the first report of rotavirus P[9] in Vietnam and suggesting that animal-like rotavirus infection in human beings.

Clinical manifestations and management of peptic ulcer diseases in children at No 1. Children Hospital from June 2013 to January 2014

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Objectives: since 2011, guilines of NASPHGAN and ESPHAN, "test and treatment" strategy is not recommended in children, eradication HP is focus in the presence of Hp-postive peptic ulcer diseases(PUD) . This aim of this study was to describe clinical, paraclinical presentation of PUD, in which to pay special attention to antibiotic resistance ratio of HP.

Methods: All children were diagnosed PUD by endoscopy. Cause of PUD is based on positive histology findings. HP determination if histology is negative, must be determinate by rapid urease test or HP antigens in stool or UBT. All children is treated by triple therapy: 1PPI+2antibiotic. Medical history, clinical features and following efficacy of the first-line therapy in HP eradication in all these children is noted by data form. If failure with the first-line therapy, children had been by the second upper endoscopy to confirm ulcer statis, HP determination, culture and antibioticl resistance to HP.

Results: From 762 endoscopy examinations, 73 children with 71 duodenal ulcer and 1 gastric ulcer, 1 case of both. They were mean 11 years old, 84.9 % cases were boy. Almost cases (64.4 %) are admitted with upper gastrointestinal bleeding, 27% cases with abdominal pain. 95.9 % of them has HP infection in histology. There were 54.8 % of cases were failed for eradication after first-line treatment. Among of those patients, the in vitro resistance rates of HP to Clarithromycin, Metronidazole, Tetracycline, Amoxicillin và Levofloxacin were 86.5%, 59.5%, 27%, 21.6% and 27%, respectively. **Conclusions**: Peptic ulcer disease almost is duodenal ulcer. Cause of PUD is Helicobacter pylori. Failure with first-line HP eradication is 54.8 % peptic ulcer patients. Among patients who fail with first triple therapy, the in vitro resistance rate to antibiotic of HP were very high.

Cosavirus in Humans and Pigs

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Cosavirus is a newly identified virus in the family *Picornaviridae*. This virus has been detected in stool samples of children with diarrhea or non-polio acute flaccid paralysis worldwide. In this study, cosavirus was detected in a fecal sample of pediatric patient with acute gastroenteritis (AGE) in Japan. Furthermore, the virus was detected in the stool samples from pigs with and without diarrhea in Thailand and Japan.

A total 630 stool samples were collected from children with AGE in 6 pediatric clinics, in Japan, between November 2011 and April 2012. Two hundred forty seven stool samples were collected from pigs with and without diarrhea, in Thailand and Japan. The detection of cosavirus was conducted by RT-nested PCR, targeting the 5'-UTR region of the viral genome.

Cosavirus was found in a single sample (10928/JPN) from a child aged six month. This sample was negative for other common 12 diarrheal viruses. The clinical feature of patient was severe diarrhea, and no other findings including vomiting, fever and upper respiratory symptoms. Analysis of complete coding-sequence revealed that the strain was classified as a new genotype of cosavirus species A. The virus copy numbers of the clinical samples was found to be 2.85×10^7 copies/g of stool. The pigs with and without diarrhea, had high positive rates (18.9 to 58.1%) for the virus. However, their virus concentrations were very low, when compared with that of the human clinical sample.

Cosavirus has been found in stool samples of both diarrhea as well as healthy individuals, and the pathogenicity of cosavirus remains to be uncertain. In this study, cosavirus strain 10928/JPN was isolated from a pediatric patient with AGE, and other common diarrhea-caused viruses were not found in the sample. Therefore, cosavirus seemed to be the viral agents that cause AGE in this patient. In addition, cosavirus was detected in the stool samples from both healthy and diarrheal piglets. These data demonstrated that pigs are one of the important hosts of cosavirus and the zoonotic transmission from pigs is likely to cause disease in humans.

Searching for the host factors involved in hepatitis E virus infection

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Hepatitis E virus (HEV) has caused acute and fulminant hepatitis E even in developed countries. Using PLC/PRF/5 cells and HEV, an efficient propagation system was recently established. Since PLC/PRF/5 showed the limited permissiveness for HEV infection, the single-cell cloning was performed and characterized. Notably, the replication efficiencies measured by transfection of an infectious clone did not correlate with the permissiveness. However, hepatitis E virus-like particles (HEV-LPs) demonstrated diverse binding level to these nonpermissive subclones, suggesting that these subclones have some deficiencies in the attachment and entry steps of HEV infection. For searching the causative host factors, we established nonadherent cell line (P3U1, binding of HEV was not observed) in which cDNA from permissive subclone to HEV was integrated. If host factor candidates were integrated, these cell lines formed colonies on HEV-coated plate. By using this panning method, one candidate was suggested. We obtained several cell lines that stably expressing this candidate; a nonadherent cell line (expression level of the candidate was confirmed to be low) and 4 nonpermissive PLC/PRF/5 cell lines. Nonadherent cells stably expressing the candidate allowed attachment of HEV. If nonpermissive PLC/PRF/5 cell lines stably expressing the candidate were infected with HEV, one cell line showed expression of HEV antigen by flow cytometric analysis although infection of HEV was not confirmed by ELISA of HEV antigen and real-time PCR of HEV genome. This phenomenon suggested that this subclone has several deficiencies not only the candidate but also the other factors for the recovery of normal virus production. Investigation of the other stable subclones is in progress.

This study is collaboration with Drs. Tian-Cheng Li, Sayaka Yoshizaki, Yorihiro Nishimura, Hiroyuki Shimizu, Masayuki Shimojima, Masayuki Saijo, Takaji Wakita, and Koji Ishii.

Molecular Characterization and Zoonotic Potential Analysis of Rotaviruses Detected in Children and Piglets with Diarrhea

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Rotaviruses are the major cause of severe acute gastroenteritis in young children and in young animals of many species, especially in pigs. The objectives of the present study aimed to assess the prevalence and to perform molecular characterization of human and porcine rotaviruses with diarrhea. A total of 401 stool specimens were collected from children hospitalized with diarrhea in Chiang Mai, Thailand during January 2013 through February 2014. In addition, 491 stool samples were collected from diarrheic piglets in several pig farms in Chiang Mai and Lamphun provinces during January 2011 to March 2014. All stool samples were screened for group A rotavirus by reverse transcription-polymerase chain reaction. Group A rotaviruses were detected in 137 out of 401 (34.2%) and 113 out of 491 (23.0%) stool specimens collected from children hospitalized with diarrhea and diarrheic piglets, respectively. For human rotaviruses, G3P[8] was the most predominant genotype (49.6%), followed by G1P[8] (23.4%), G2P[4] (13.9%), G1P[4] (4.4%), G8P[8] (2.9%), G2P[8] and G9P[8] (each of 2.2%), and mix-infection of G3 in combination with P[8] and P[4] (0.7%). Interestingly, the uncommon strain of human rotavirus G9P[19], was detected in a child with diarrhea in this study. The genetic sequence analysis of VP7 and VP4 genes of G9P[19] rotavirus demonstrated that the virus isolated from human was more closely related to the porcine rotavirus. For porcine rotaviruses, G4P[13] was the most prevalent genotype (29.2%), followed by G4P[23] (14.1%), G5P[23] (11.5%), G4P[6] (9.7%), G3P[23] (7.0%), G5P[13] (6.1%), G3P[13] (4.4%), G3P[6] and G5P[6] (each of 2.7%). In conclusion, the present study provides valuable epidemiological information and molecular characteristics of rotavirus strains circulating in pediatric patients and in piglets with acute gastroenteritis in Chiang Mai and Lamphun provinces. Thailand.

Structural analysis of histo-blood group antigen binding specificity of norovirus variants

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Introduction: The outbreak of acute gastroenteritis due to norovirus occurred and continues in many places since 2012. In this study, we described surveillance study of norovirus infections among Japanese pediatric outpatients and investigated the antigenic change and binding patterns of norovirus GII.4 variant.

Materials and Methods: A total of 2,908 fecal specimens collected from children with acute gastroenteritis in Japan from 2009 to 2014 were examined for norovirus. The genotypes were further analysed by sequence analysis. Binding specificity of norovirus GII.4 variant was determined by an ELISA and immunostaining using blood group substances.

Results: Norovirus was detected in 43.0% with several genotypes. Interestingly, the Sydney_2012 variant was found from mid-2012. Analysis of P2 subdomain showed a high level of diversity in the antigenic sites and HBGA binding sites. The Sydney_2012 showed the strong binding to secretor individuals irrespective of ABO and Lewis phenotypes, but absence of clear binding to non-secretor.

Discussion: Analysis of noroviruses circulating in the past 5 years revealed a change of the predominant variant in each epidemic season over time. The changes of binding specifities occured in GII.4 variants. It was suggested that the expression of a1,2-fucosylated glycan is crucial for susceptibility to infect with Sydney_2012.

Rotavirus infection in the transitional period of rotavirus vaccine implementation

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Introduction: World Health Organization recommends all the nations to include rotavirus vaccines in the national immunization program. In Japan, they have been introduced as optional vaccinations since November 2011. In this study, we investigated the cumulative vaccine coverage rate in one city in Tokyo to see how widely they have been used. In addition, we described surveillance study of rotavirus infections among Japanese pediatric outpatients to investigate the molecular epidemiological trends during the transitional period of rotavirus vaccine implementation. Materials and Methods: Vaccine histories were investigated with a total of 376 mother and child health handbooks of children at 18-month well-child visits in Fuchu-city. Tokyo, Japan in September and October 2013. A total of 1857 fecal specimens were collected from children with acute gastroenteritis in Japan from 2011 to 2014 to be examined for rotavirus. The G and [P] genotypes were further analyzed by multiplex PCR and sequence analysis. VP6 genotypes of G1P[8] strains were determined by genotype specific primers to see the prevalence of reassortant strains. **Results**: The vaccination rate of rotavirus vaccines was 33.4% (95%CI:±4.6%). The number of children vaccinated was significantly smaller with rotavirus vaccines than with universal vaccines. Moreover, significantly fewer children were vaccinated against rotavirus than against Hib and varicella. During 2011 to 2014, detection rate of group A rotavirus declined dramatically $(17.9\% \rightarrow 22.1 \rightarrow 3.8\%)$. Prevalence of G1 strains declined from 52.8% (2011-2012) to 30.0% (2013-2014) and that of G2 increased from 0% (2011-2012) to 25.0% (2013-2014). DS-1 like G1P[8] reassortant strains were detected at significant level during these three years $(35.0\% \rightarrow 89.4\% \rightarrow 66.7\%)$. **Discussion**: The lack of financial support and disease recognition may have resulted in the low vaccination rate of rotavirus vaccines. Possibly due to herd immunity effect, the detection rate of group A rotavirus declined during the observed period. It is warranted to continue monitoring the selective pressure against G2 strains and the spread of reassortant strains like DS-1 like G1P[8] strains.

This study is the collaboration with Drs. Aksara Thongprachum, Shoko Okitsu, Mana Inoue, Hiroshi Sakiyama, Masashi Mizuguchi, Satoshi Hayakawa, and Hiroshi Ushijima.