Online ISSN 2424-0907
Print ISSN 2189-5171

ADC Letter
for Infectious Disease Control

Vol.4 No.2 2017.7.1

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/
J-STAGE: https://www.jstage.jst.go.jp/browse/adc

TEIKYO

CONTENTS

EDITORIAL

ADC Letter Vol.4 No.2をお届け致します。

・フィリピンとの共同臨床試験がスタート

長年の準備を重ねた1つのゴールでもありました。この臨床試験を進めるにあたっては、JST-AMEDの支援によるe-ASIA JRPのプロジェクトではじめて実現し、3年間の区切りとなりました（pp.26–28）。

・日本バイオイメージング学会国際シンポジウムに参加

シンガポール大学と日本バイオイメージング学会との共催でシンガポールを訪問し、現地のAMEDや日本大使館のみなさんと交流をしました（pp.37–40）。

・帝京大学医学部5年生の「ベトナム感染症実習」

昨年からスタートしました帝京大学医学部5年生の「ベトナム感染症実習」は、好評で、今年も実施します（p.48）。

Presentation of
Ms. Nguyen Thu Thuy

・研究所での研究のトピックスとしては・・・

文科省の国費留学生で2014年10月に大学院医学研究科に入学しましたNguyen Thu Thuyさんが、この秋4年生になるので、研究の中間発表を行いました。文化、生活習慣、環境などが全く違い苦労したようですが、研究成果が着々とではじめ、ラストスパートに向けて励んでいます（p.29）。

I am delighted to issue the ADC letter for infectious disease control: Volume 4 No.2.

Joint clinical trial with Philippines (pilot study)

It is noteworthy that a joint clinical trial with the Philippines began in March after long preparation period. This clinical trial is carried out by e-ASIA JRP project supported by JST-AMED (pp.26–28).

The 5th International Symposium for Bioimaging in Singapore

In May, Bioimaging Society and National University of Singapore held the 5th International Symposium for Bioimaging in Singapore (pp.37–40).

"Infectious Diseases Training in Vietnam" for Medical Students of Teikyo University

Training in Infectious Diseases in Vietnam for 5 years grade of medical students in Teikyo University gained good reputation, and some students are going to participate the training this year (p.48).

Research topics in ADC

Ms. Nguyen Thu Thuy, Ph.D. student, reported her current study (p.29).

e-ASIA JRP のまとめ

e-ASIA JRP: Japan-Vietnam-Philippines

Pulmonary Infectious Diseases induced associated with Influenza and TB in Asia Countries

JAPAN(AMED)

Leader:
Prof. Kazuo Suzuki,
Teikyo University

VIETNAM(MOST)

Leader: Dr Phung Thi Bich Thuy,
Laboratory Chief, National Children's Hospital, Hanoi

PHILIPPINES(DOST)

Leader: Prof. Jaime Montoya
Professor, University of the Philippines, College of Medicine, Manila

All member's meeting at Tokyo and Hanoi

e-ASIA Joint Research Program
AMED(JST)-MOST-DOST

2014 - 2017

1	2014.1.16-19	Kick-off (Tokyo)
2	2014.1.19	Symposium (Tokyo)
3	2014.3.3-6	Annual Meeting (MOST-NHP, Vietnam)
4	2015.1.16-17	Annual Meeting (Tokyo)
5	2015.10.12	Project Progress (Bangkok, Thailand)
6	2015.11.9-10	Annual Meeting (NHP, Vietnam)
7	2016.2.25-27	Progress Meeting (NHP, Vietnam)
8	2016.2.28-3.2	Progress Meeting (RITM, Philippines)
9	2016.5.2-6	Progress Meeting (NHP, Vietnam)
10	2016.6.22-25	Progress Meeting (RITM, Philippines & NHP, Vietnam)
11	2016.10.17-19	Annual Meeting (RITM & Tagaytay, Philippines)
12	2017.1.22-23	Protocol Preparation (UP-PGH, Philippines)
13	2017.2.19-22	Progress Meeting (NHP, Vietnam)
14	2017.3.12-14	Josamycin Transfer (UP-PGH, Philippines)

Flu Team : フィリピンでの臨床試験
Clinical Trial with Philippines

March 14th, 2017

Drs. Kazuo Suzuki, Sally Gatchalian and Shoji Kawachi

Receive of Josamycin

at

Department of Pediatrics
Philippine General Hospital, University of the Philippines/Manila

TB Team：結核菌分子疫学研究フィールド フィリピン / ベトナム
TB Molecular Epidemiology Study in Philippines/Vietnam

San Juan, Batangas, the Philippines

Every year, ~500 individuals visit health care units for possible TB

Recruitment

- Sputum smear-positive pulmonary TB patients
- Number of patients: 213
- Culture-positive: 192
- Demographic information has not been summarized yet.
- Reculture of MTB isolates ongoing

Da Nang city, Vietnam

Every year, 700 to 800 new TB patients are reported in the entire city

Recruitment

- Sputum-smear positive active pulmonary TB (new cases: 85%)
- Number of patients: 251
- Sex: male 80%
- Peak age at onset: ~40 y.o.
- Reculture and DNA extraction of MTB isolates ongoing

鈴木和男：ベトナム RICH-NCH 客員教授に
Prof. Kazuo Suzuki: Visiting Professor in RICH-NCH, Vietnam

Vietnam National Children's Hospital

RESEARCH INSTITUTE FOR CHILD HEALTH (RICH)

bestows the title

Visiting Professor

upon

Professor Kazuo Suzuki

Teikyo University, Japan

for the time period

16 November 2016 to 16 November 2021

In order to take part in the education and training activities and to promote collaboration between RICH and distinguished experts within an international network of excellence

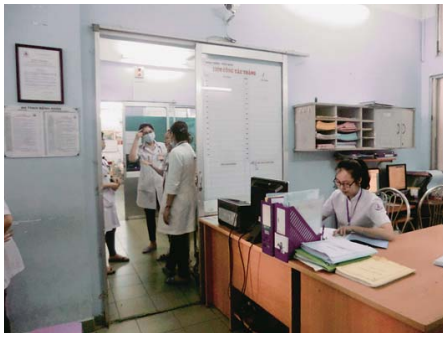
A/Prof. Le Thanh Hai

Director, Research Institute for Child Health

Visiting National Children's Hospital-1, Ho Chi Minh City

February 21th, 2017

Drs. Dao Trung Hieu, Kazuo Suzuki, Truong Huu Khanh, Nguyen Minh Tuan, Nguyen Ngoc Tu Anh and Nguyen Dong Bao Chau



2017年6月24日医学研究科学位論文中間発表会が板橋キャンパス大学棟1階で開催されました。ADC研究所に所属しているベトナムからの国費留学生Nguyen Thu Thuyさんが研究の中間発表を行いました。

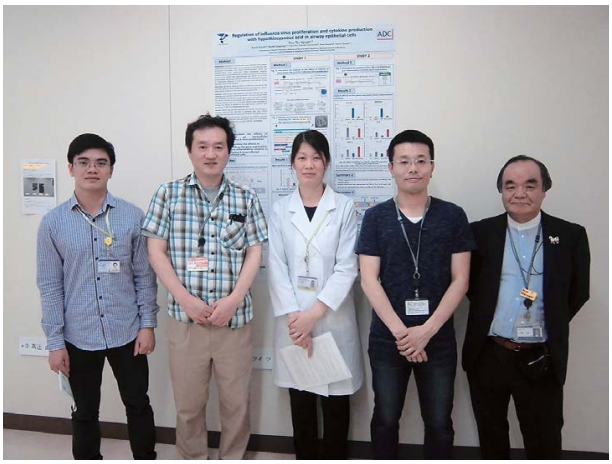
Mid-term Doctorate Presentation

大学院医学研究科　3年　Nguyen Thu Thuy

I have studied Ph.D. program in Teikyo University for 3 years. My study is carried out at Asia International Institute of Infectious Disease Control (ADC). In the research project during my study on regulation of influenza viral infection, we made a poster presentation for current activity of Ph.D. study: “Regulation of Influenza virus proliferation and cytokine production with hypothiocyanous acid in airway epithelial cells” on July 24, 2017. We will clarify the mechanism of these results and hypothesis in the future. We hope that we can get the good results to complete our study.

I would like to thank my supervisor, Professor Kazuo Suzuki, for his guidance, support and patience during my research. I am grateful to my advisor, Dr. Shoichi Suzuki, for his direct guiding in the lab, proving me the valuable comments and new ideas of my work. I also want to say thank all the members in ADC Institute for their help and good suggestion.

This research project is supported by Japanese Government Scholarship Monbukagakusho (MEXT) and e-ASIA Joint Research Program, Japan Agency for Medical Research and Development (AMED), Japan Science and Technology Agency (JST).



Tran Huu Dat、鈴木章一、Nguyen Thu Thuy、
菅又龍一、鈴木和男

発表風景

Research Progress in 2017FY

2017年度　研究プログレス

1-1. ヒポチオシアン酸による気道炎症
　　鈴木章一（講師）「文科省：科研費基盤C採択」
1-2. Roles of NS1 in influenza virus infection
　　Nguyen Thu Thuy（大学院医学研究科3年）
2-1. Development of novel anti-influenza A virus drug based on 16-membered macrolide derivatives
　　菅又龍一（助教）「文科省：科研費若手B採択」
2-2. The inhibitory activity of macrolide derivatives in proliferation of 2009 pandemic influenza A/H1N1 viruses
　　Tran Huu Dat（大学院医学研究科2年）
3-1. 抗インフルエンザ薬治療が不良な小児の原因解析（帝京大学医学部小児科および徳島大学との共同研究）
3-2. MPO-ANCA関連血管炎におけるモエシンの発現解析（厚生労働省研究班：協力者）
　　伊藤吹夕（研究助手）

好中球研究の思い出

愛泉会日南病院　院長　布井　博幸
（元：宮崎大学医学部発達泌尿生殖医学講座小児科学分野　教授）

Memories of My Phagocyte Studies

Hiroyuki Nunoi, M.D., Ph.D.
Director of Aisenkai Nichinan Hospital
Former Professor of Division of Pediatrics, University of Miyazaki

Phagocytes had been very difficult to deal with, because they have a small number in the peripheral blood and short life span. However, neonatal phagocyte screening with TAXscan in addition to NGS (next generation sequencer) will make it simple and precise to find their functional and molecular background and new aspect of phagocyte disorders will be explored in a near future.

はじめに：私は熊本大学を1977年に卒業し、研修医、大学院を卒業後、1986年から1989年まで米国立衛生研究所の細菌感染研究室（Harry L Malche室長）で3年半慢性肉芽腫症（Chronic Granulomatous Disease; CGD）の研究をさせて貰い、その当時としては大きな成果だったかもしれませんが、一つの時代だったのだなと感じました。これまでの好中球研究は、抹消好中球数が少なく、好中球の寿命も短いことから、なかなか進みませんでした。そこに至る過程、それから現在までで感じたことを徒然なるままに書かせていただきます。しかし、TAXScanなどの新しい機器により、少量で正確な機能検査が可能となり、好中球の新たな疾患や分子機構が明らかになるのではないかと期待しています。

1970年代熊本大学では好中球やリンパ球などの白血球の走化因子と種々の炎症との関係を実験病理の林秀夫教授以下多くの先生が研究されており、大学の授業でも色々な炎症の話をされていて、実際の病気ではどうなっているのだろうと興味をもたせてもらいました。1977年に熊本大学で小児科に入局し、はじめのころ診せてもらった患者さんが肺にcold abscessを形成し、血清IgEが20,000単位/μlを超えるHyper IgE症候群と診断された可愛い女の子でした。この子を長く診ることが出来たのは大変勉強になりました。はじめ、血清中に好中球の遊走能を抑える物質が有るのではないかという結果となり、血漿交換を何度もやったことを覚えています。その後同様な結果が出た患者さんもおられ、血漿交換後は経過も悪くなく、軽快してくれていましたので、意を強くしていたのです[1]。しかし、その後、この病気の本体がSTAT3の遺伝子異常が明らかになり、副次的な反応を追いかけていたのだと反省しました。患者はその後20歳で肺がんを発症され、内科のお世話になりましたが、若くして亡くなられました。このことが、最後まで論文にできず、残念でもあり、申し訳なく思っています。

この間、研修中の病院で出会った患者さんについて述べますと、Leukocyte Adhesion Deficiency（LAD）と後で診断できた症例があります。はじめの姉については緑膿菌性肺炎があり、訳も判らず亡くなってしまわれました。申し訳なくて、剖検をお願いしましたが、母親には断られました。しかし、父親がタクシーまで行ったところで、「先生勉強しろ」と言って、患児を渡して頂きました。有難く、必死で文献もしらべましたし、病理の先生方にも色々助言を頂いたことを覚えています。1年もしないうちに、このLADの論文が目に入りました。疾患が鑑別に入られると好中球遊走能、粘着能、FAX解析と順調に解析が進み、日本でも10例以下ですが、症例が集積され、色々な遺伝子変異の患者がおられることがわかりました[2]。その時の病理についても報告させてもらいました[3]。

鈴木先生と一緒に調査・解析させて頂いた疾患がMPO欠損患者さんでした。はじめは、患者のろ紙血からMPOを抽出してそれを抗体で定量してもらっていました。後には、好中球のMPO活性染色して、好中球をカウントできるTechnicon H（Bayel-Sankyo, Co., Tokyo）で、スクリーニングされたMPO欠損患者を、各々の施設から報告いただき、日本全体での患者を割り出すことが出来ました[4]。MPO欠損患者と後に述べるCGD患者の違いを実感する良い機会をいただきました。

その後に診させてもらった患者さんもその原因を明らかにするのに、大変苦労しました。患者さんに出会ったのは1980年はじめで、私が大学院生の頃でした。易感染性があり、巨核芽球を伴う血小板減少症（8-10万/micro l）、好中球減少症？（常に桿状核球が分葉核球より多い状態）、日光過敏症と思われる皮疹、軽度の知能低下があり、精査するように言われました。好中球の活性酸素産生能、遊走能を色々な刺激（fMLP, Zymosan, PMAで計測しましたが、どれも低下しており、何らかの異常があるに違いないと思いましたが、まだ好中球の分離技術が下手ではないかとか考え、細胞膜電位という他の2つよりより刺激に左右されない計測をしてみましたが、脱分極も再分極も反応が遅く、何らかの異常があると確信しました。他の手段で何か見つからないのかと考え、その頃よくやっていた細胞の二次元

電気泳動で、正常細胞と比べてみました。その結果、何と細胞質のmajorタンパク質であるアクチンのスポットのより塩基側に同様の大きな蛋白スポットが認められました（図1）。きっと細胞質アクチンに違いないと抗体を用いて確認に入りましたが、β-, γ-アクチンの区別をつけられる抗体が見つからず、挙句にはmRNAを読んでみると偽遺伝子が多く、何を読んでいるのかさえわからない有様になってしまいました。しかし、1980年後半は、まだそのペプチドのアミノ酸を読む技術が十分でなく、大量の蛋白を集めないと解析できないことがわかり、ペプチドレベルでの確認を諦めざるをえませんでした。1990年後半になり相模原遺伝研究所の加藤誠志部長の開発された蛋白発現ベクターを使わせていただき、異常なクローンが確認でき、遺伝子レベルでも、更に、そこから考えられたprofilinとの結合力の弱さも確認できるようになりました[5]。この細胞アクチンの異常症については、それ以降、2012年までは報告がなく、不安でしたが、Nature Geneticsに18例の報告がされました。やはりphenotypeは違っても世界から見ると、このような疾患があるのは当然だと思いました。

1998年頃ですが、インフルエンザ脳症についての出会いました。血中にはインフルエンザのウイルスは認められないのに脳症が起こること自体不思議でした。肺と脳組織をつなぐ炎症物質と考えたときに、好中球と種々のサイトカインが絡むと思っていましたが何も見つけられませんでしたが、1999年頃、結果として血中のcytochrome cが非常に上昇し、apoptosisが誘導されているらしいことは多くの症例の解析でわかりました[6]。ステロイドのパルス療法の早期導入でかなり軽症化できることもわかりました。その後、同じインフルエンザによる脳症にもその病因として、サイトカインストームによるMSES, ANEや、興奮毒性によるてんかん重責AESD, HHE、代謝異常症CPTII欠損、MAT1欠損、その他のAERRPS, MERSなど脳梁膨大部浮腫を伴う脳症などが分類されるようになりました（図2）[7]。脳症のことをやっていましたので、2006年ころから鈴木先生方とインフルエンザ感染による重症ARDSについてもベトナム・ハノイの国立小児病院での調査研究に参加することができました。インフルエンザによる重症ARDSはインフルエンザ感染後1週間までは呼吸器症状もそこまで酷くないのですが、1週間を過ぎた頃から急速な呼吸不全に陥り、死亡される方がおられること（図3）、そこにはCoxsackie virusやEntero Virusの重感染も起こっているらしいことなど多くの治験を得ることが出来ましたが、まだまだ症例の積み重ねが必要な気がしました。

1986年9月から米国立衛生研究所（NIH）では慢性肉芽腫症の細胞質因子に関する研究をさせていただきました。そこでは活性酸素産生酵素複合体の細胞質因子の関与について、Cell free superoxide産生系を駆使して、常染色体劣性遺伝型のCGD患者の解析をしました。まず関連する細胞質蛋白分画が取れ、抗体ができ、遺伝子が取れ、患者の解析がそれまで米国NIHに集積されていた症例を中心に蛋白、遺伝子解析が行われ、慢性肉芽腫症の全体像が明らかになってきました（図4）[8,9]。NIHで驚いたのは、好中球のアフェレーシスをしていただけるボランティアの方が

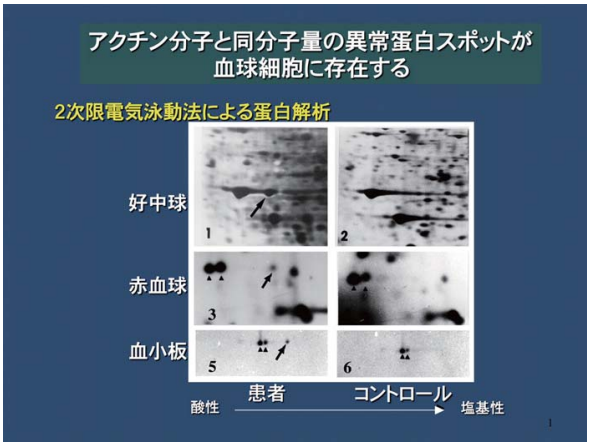


図1

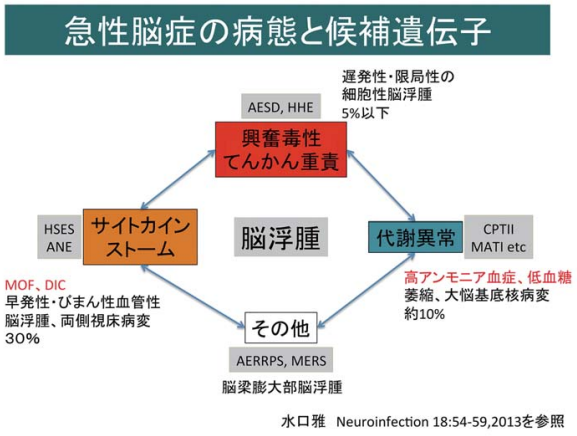


図2

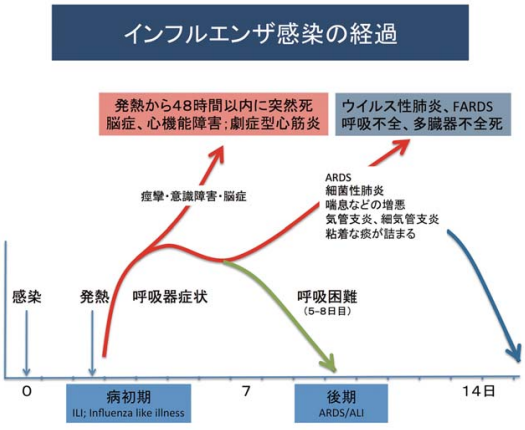


図3

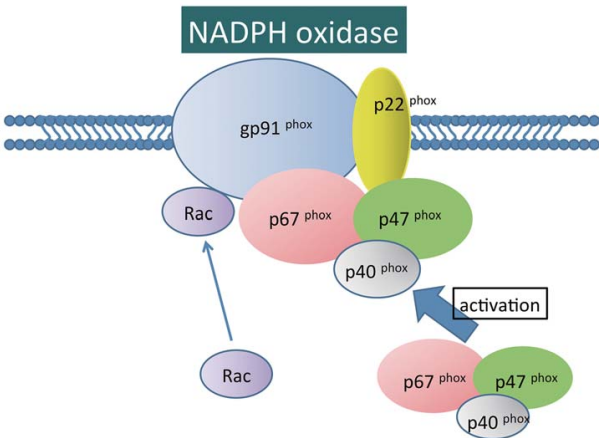


図4

中近東・東南アジアとの比較

	USA Winkelstein,2000	Europe van Berg,2009	Turkey ESID 2016	India ESID 2016	Japan ESID 2016
n（%）	321	392	133	82	287
XL-CGD gp91^{phox}	259 (81%)	290 (74%)	50 (38%)	21(25%)	221 (78%)
AR-CGD p22^{phox}	7 (2%)	22 (6%)	32 (24%)	15(18%)	18 (6%)
p47^{phox}	45 (14%)	69 (18%)	32 (24%)	41(50%)	17 (6%)
p67^{phox}	10 (3%)	11 (3%)	19 (14%)	5 (6%)	27 (10%)
unknown	47	37		23	92

図5

おられ、毎週大量の好中球を容易に準備できたことです。日本では、豚の血を頂いていたこともあり、医科学に対する考え方が市民レベルで違うのだと、NIHの底力を知りました。一方で先端技術といっても、やっとPCRがNIHでできるようになったのが1988年くらいでしたし、日本と技術の差としてはあまり感じませんでした。日本に帰ってからは、日本の慢性肉芽腫症患者の登録事業を受け持たせてもらい、現在まで400名に及ぶ解析をしました。そこで、欧米と比較するとp47phox欠損患者が明らかに日本では少ないことに気づき[10]、どうなっているのかを考えていた2010年はじめになると世界中で遺伝子解析も可能になり、p47phox欠損型CGDがトルコなど中東からインドにかけて慢性肉芽腫症の30－50％を占めているという、非常に偏った遺伝型を示していたことが報告されてきました。遺伝子変異の偏在や血族結婚などの環境要因が必ずや関わっていると思われ、このような疾患に対するアプローチについては、やはりGlobalな調査がどうしても必要なことが分かってきました（図5）。一方で、遺伝子検査が非常に進み、今考えると、CGDも適切な家系例のexome解析をしたら、当然明らかになっていたことだろうと思うと、それまでの苦労が、苦労でしかないような時代になってきているのだと、痛感させられました。遺伝子解析はそれとして、治療については、喜びと落胆の連続でした。新しい医療技術を開発することの難しさ（倫理面、技術面、副作用のモニター、市民レベルでの啓蒙と理解）を感じています。しかし遺伝子編集技術も含めて大きな可能性を感じている次第です。

主に好中球に関わる異常症の研究については、血球の寿命が短いこと、機能解析に細胞数が必要なこともあり、1970年代の研究は、炎症をやっと物質のレベルで語ろうとする時代でした。その後モノクローナル抗体、細胞株の樹立、そして1990年代になると、遺伝子ハンターが闊歩し、2010年代にはNGSによるwhole exome解析と、遺伝子レベルで語れる、否、語らないといけない時代になりました。一方でごく少量でしかも経時的な好中球の機能を記録できる装置（TAXScan）が考案され、好中球機能の新生児スクリーニングですら可能な時代になろうとしています。解析技術はどんどん進みますし、時代の波を感じる力と患者の本当のことを知り、何とか全体として理解しようという情熱で研究をする時代になるだろうと思います。ユヴァル・ノア・ハラリも書かれているように、ホモ・サピエンス（賢いヒト）が積み重ねてきた歴史からしても、我々は色々な人と知り合い、語り合い、一歩一歩を踏みしめて、飛躍的な科学技術の進歩は起こっていますが、これらを受け止め、どのように活かしていくか、（過去の思い出に浸りやすいという）我々の心の有り様に、遅れを感じています。

1) Chikazawa S, Nunoi H, Endo F, Matsuda I, Honda M. Hyperimmunoglobulin-E-associated recurrent infection syndrome accompanied by chemotacticinhibition of polymorphonuclear leukocytes and monocytes. Pediatr Res. 1984; 18(4): 365-9.
2) Matsuura S, Kishi F, Tsukahara M, Nunoi H, Matsuda I, Kobayashi K, Kajii T. Leukocyte adhesion deficiency: identification of novel mutations in two Japanese patients with a severe form. Biochem Biophys Res Commun. 1992; 184(3): 1460-7.
3) Nunoi H, Yanabe Y, Higuchi S, Tsuchiya H, Yamamoto J, Matsuda I, Naito M, Takahashi K, Fujita K, Uchida M, et al. Severe hypoplasia of lymphoid tissues in Mo1 deficiency. Hum Pathol. 1988; 19(7): 753-9.
4) Nunoi H, Kohi F, Kajiwara H, Suzuki K. Prevalence of inherited myeloperoxidase deficiency in Japan. Microbiol Immunol. 2003; 47(7): 527-31.
5) Nunoi H, Yamazaki T, Tsuchiya H, Kato S, Malech HL, Matsuda I, Kanegasaki S. A heterozygous mutation of beta-actin associated with neutrophil dysfunction and recurrent infection. Proc Natl Acad Sci U S A. 1999; 96(15): 8693-8.
6) Nunoi H, Mercado MR, Mizukami T, Okajima K, Morishima T, Sakata H, Nakayama S, Mori S, Hayashi M, Mori H, Kagimoto S, Kanegasaki S, Watanabe K, Adachi N, Endo F. Apoptosis under hypercytokinemia is a possible pathogenesis in influenza-associated encephalopathy. Pediatr Int. 2005; 47(2): 175-9.
7) Hoshino A, Saitoh M, Oka A, Okumura A, Kubota M, Saito Y, Takanashi J, Hirose S, Yamagata T, Yamanouchi H, Mizuguchi M. Epidemiology of acute encephalopathy in Japan, with emphasis on the association of viruses and syndromes. Brain Dev. 2012; 34(5): 337-43.
8) Nunoi H, Rotrosen D, Gallin JI, Malech HL. Two forms of autosomal chronic granulomatous disease lack distinct neutrophil cytosol factors. Science. 1988; 242(4883): 1298-301.
9) Clark RA, Malech HL, Gallin JI, Nunoi H, Volpp BD, Pearson DW, Nauseef WM, Curnutte JT. Genetic variants of chronic granulomatous disease: prevalence of deficiencies of two cytosolic components of the NADPH oxidase system. N Engl J Med. 1989; 321(10): 647-52.
10) Ishibashi F, Nunoi H, Endo F, Matsuda I, Kanegasaki, S, et al. Statistical and mutational analysis of chronic granulomatous disease in Japan with special reference to gp91-phox and p22-phox deficiency. Human genetics 106.5 2000; 473-81.

The 18th International Vasculitis & ANCA Workshop

−Diversity and Integration for Tomorrow−

25 (Sat)-28 (Tue) March, 2017

Ito International Research Center, The University of Tokyo, Japan

18回目に日本で初めてInternational Vasculitis & ANCA Workshopが開催された。

これに先立ち、5年前に【The Asia-Pacific Meeting of Vasculitis and ANCA Workshop, 2012】が、21か国469人の参加を得て品川で開催された。鈴木和男が会長をつとめ、ちょうど帝京大学に赴任する直前であった。

これは、今回の国際会議を日本で開催するPre-Meetingとして、海外のメンバーが多数発表・参加して、第18回International Vasculitis & ANCA Workshopを行うことにつながった。

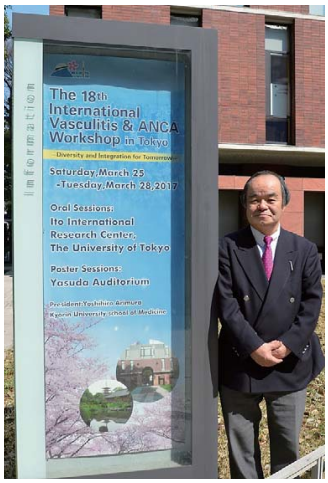
今回の国際会議は、前回の第17回ロンドン大会で、国際委員の懇親会場で、David Jayneらから私に次回の日本での開催についての打診があり杏林大学の有村先生を推薦した。翌日、有村先生が会長として受け入れることを内諾し、委員会にて正式決定となった。第18回のオーガナイザーは、以下のメンバーで構成され、最終的に39か国、738人の参加を得て、これまでにない盛大なプログラムによる会議となった。

日本からも多くの発信があり、臨床、疫学、基礎の研究発表と討論があった。

The 18th International Vasculitis & ANCA Workshop was held in Japan for the first time. Prior to it, five years ago [The Asia-Pacific Meeting of Vasculitis and ANCA Workshop, 2012] was held in Shinagawa with 469 attendees from 21 countries. Kazuo Suzuki organized the meeting as a president, just before his being assigned to Teikyo University. Many overseas attendees made presentations in the meeting, and the event became the basis of the 18th International Vasculitis & ANCA Workshop.

Prof. David Jayne in Addenbrooke's Hospital, Cambridge University and Chairman of EUVAS, suggested me about the next meeting in Japan at the reception party for excutine committee members of the 17th meeting in London. I recommended Prof. Yoshihiro Arimura in Kyorin University to be the president of the 18th meeting in Japan. He accepted it and it was officially decided at the committee. The 18th International Vasculitis & ANCA Workshop organizers were consisted of the following members, with 738 attendees from 39 countries. It was a conference with a wonderful program.

There were also a lot of clinical, epidemiological and basic research presentations and discussions from Japanese and overseas.



Scientific Programming and Organizing Committee

President: Yoshihiro Arimura (Kyorin University School of Medicine)

Domestic committee

Chair of Organizing Committee: Shoichi Fujimoto (University of Miyazaki)

Chair of Programming Committee: Akihiro Ishizu (Hokkaido University)

Special Committee: Kazuo Suzuki (Teikyo University)

Yasuaki Aratani (Yokohama City University)

Takao Fujii (Wakayama Medical University)

Yasuaki Harabuchi (Asahikawa Medical University)

Masayoshi Harigai (Tokyo Women's Medical University)

Junichi Hirahashi (Keio University)

Shunsei Hirohata (Kitasato University School of Medicine)

Sakae Homma (Toho University School of Medicine)

Toshiko Ito-Ihara (Kyoto University Hospital)
Kensuke Joh (Tohoku University)
Shinya Kaname (Kyorin University School of Medicine)
Takashi Kanda (Yamaguchi University)
Soko Kawashima (Kyorin University School of Medicine)
Tamihiro Kawakami (St. Marianna University)
Yoshinori Komagata (Kyorin University School of Medicine)
Eri Muso (Kitano Hospital and the Tazuke Kofukai Medical Research Institute)
Takahiko Sugihara (Tokyo Metropolitan Geriatric Hospital)
Kei Takahashi (Toho University Ohashi Medical Center)
Yoshinari Takasaki (Juntendo University)
Kazuo Tanemoto (Kawasaki Medical School)
Masami Taniguchi (Sagamihara National Hospital)
Shunya Uchida (Teikyo University School of Medicine)
Kunihiro Yamagata (University of Tsukuba)
Hajime Yoshifuji (Kyoto University)
Wako Yumura (International University of Health and Welfare Hospital)

International committee

Ingeborg Bajema (Netherlands), Bo Basland (Denmark), Min Chen (China), Maria Cid (Spain), Kirsten de Groot (Germany), Ron Falk (USA), Franco Ferrario (Italy), Luis Felipe Flores-Suarez (Mexico), Shouichi Fujimoto (Japan), Gina Gregorini (Italy), Wolfgang Gross (Germany), Loic Guillevin (France), Marion Haubitz (Germany), Peter Heeringa (Netherlands), Julia Holle (Germany), David Jayne (UK), Charles Jennette (USA), Cees Kallenberg (Netherlands), Ralph Kettritz (Germany), Richard Kitching (Australia), Peter Lamprecht (Germany), Carol Langford (USA), Raashid Luqmani (UK), Alfred Mahr (France), Eric Matteson (USA), Peter Merkel (USA), Patrick Nachman (USA), Pavel Novikov (Russia), John Niles (USA), Seza Ozen (Turkey), Christian Pagnoux (Canada), Charles Pusey (UK), Niels Rasmussen (Denmark), Andy Rees (Austria), Alan Salama (UK), Caroline Savage (UK), Judy Savige (Australia), David Scott (UK), Marten Segelmark (Sweden), Ulrich Specks (USA), Coen Stegeman (Netherlands), John Stone (USA), **Kazuo Suzuki (Japan)**, Jan-Willem Cohen Tervaert (Netherlands), Vladimir Tesar (Czech Republic), Augusto Vaglio (Italy), Michael Walsh (Canada), Kerstin Westman (Sweden), Cornelia Weyand (USA), Jorgen Wieslander (Sweden), Veronique Witko-Sarsat (France), Ming-Hui Zhao (China)

Program

Welcome Lecture

Diversity and Integration for tomorrow
Chairs: T. Nagasawa, S. Fujimoto, Speaker: Y. Arimura

Fokko Van Der Woude Lecture

Chairs: **K. Suzuki**, C. Pusey, Speaker: L. Guillevin

Shimon Sakaguchi Special Lecture

Chairs: W. Gross, Y. Arimura, Speaker: S. Sakaguchi

Symposium

1. Classification of ANCA associated vasculitis (AAV): clinical entity vs autoantibody profile
2. Kawasaki Disease: history and step to next stage
3. Pulmonary-limited Microscopic polyangiitis: a new entity of MPO-AAV or not?
4. Takayasu Arteritis and Giant Cell Arteritis: a spectrum within the same disease or not?
5. Behcet Disease
6. New drugs and Ongoing clinical trial for Vasculitis

Case Discussion

Workshop

1. ANCA and Other Biomarkers, 2. Epidemiology, 3. Genetics, 4. Molecular and Cellular Mechanisms 1, 5. Takayasu Arteritis, Giant Cell Arteritis, Kawasaki Disease, Polyarteritis Nodosa, 6. Molecular and Cellular Mechanisms 2, 7. QOL and Health Economics, 8. Head and Neck Involvement and Thrombosis in AAV, 9. Renal Pathology, Classification and Scoring

Closing Remarks: Y. Arimura

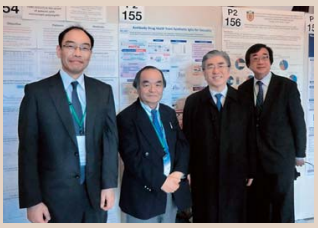
18th ANCA Workshop and Vasculitis Meeting

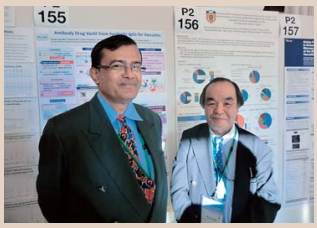
Fokko Van Der Woude Memorial Lecture
Chairs: K. Suzuki & C. Pusey
Speaker: L. Guillevin

With Prof. David Jayne
and Prof. Peter Merkel

With Dr. Tomisaku Kawasaki

At Prof. Kazuo Suzuki's Poster Presentation





第18回国際血管炎ANCA学会報告

有村義宏先生（杏林大学第一内科学教室腎臓・リウマチ膠原病内科 客員教授、吉祥寺あさひ病院 病院長）からの寄稿

2017年3月25日から28日まで、第18回国際血管炎・ANCA学会（大会長：有村義宏）を東京大学伊藤記念ホールと安田講堂で開催いたしました。おかげ様で世界39ヵ国から738名の参加があり、盛大に開催することができました。

本学会は、ほぼ2年ごとに血管炎に関する最新情報が討議される国際会議で、この分野に関しては世界で最も権威ある学会です。血管炎に関する用語、分類、診断基準、病因・病態、治療法などの最新の研究成果が論議されてきました。本学会は1988年にコペンハーゲンで第1回大会が開催され、これまで主催してきた大学や病院は、ケンブリッジ大学、パリ大学、ロンドン大学、メイヨークリニックなど世界のトップクラスの大学や病院でした。この学会を杏林大学（第一内科腎臓リウマチ膠原病内科）で開催できたことは、大変名誉なことでした。抄録集はRheumatology（Oxford）の特別号として出版されました。東洋で初めての開催のため、欧米諸国からの参加者が少なくなるのではと心配しましたが、これまでで最も多くの参加国・参加者で、44％が海外からの参加者でした。「Diversity and Integration（多様性と統合）for Tomorrow」をテーマに各国を代表する先生方、若手の医師・研究者などが集い、会場は4日間ともほぼ満席で、活発な討議が行われました。

本大会の開催は、我が国および世界の血管炎の基礎・臨床研究の進歩に貢献できたと考えています。

Visitor in ADC and Hospital, Teikyo University after 18th ANCA Workshop and Vasculitis Meeting

Francisco Silva L. M.D. M.S.
Assistant professor of Department Rheumatology
Clínica Alemana-Universidad del Desarrollo

With Staff of ADC Lab

With Director of Teikyo University Hospital
Prof. Tetsuya Sakamoto

With Dr. Hajime Kono

With Prof. Shunya Uchida

The 5th International Symposium for Bioimaging-Singapore, May 20-21, 2017 Joint Symposium on Bioimaging

鈴木 和男（帝京大学アジア国際感染症制御研究所・所長、日本側大会長）

日本バイオイメージング学会では、2017年5月20-21日、シンガポールにて、第5回国際バイオイメージングシンポジウムを開催した。シンガポール側大会長のProf. Paul Matsudairaと共に、私は、日本側大会長をつとめた。また、本シンポジウムは、早稲田大学のシンガポール研究所（WABIOS（石山敦士所長））の協賛を得て、特別講演には石渡信一先生（前WABIOS所長）とProf. Michael Sheetz（国立シンガポール大学）に講演をいただいた。シンポジウムは、シンガポール側と日本側で1つずつ、そして、「男女参画」に関する「Women in Science」のシンポジウムを日本とシンガポールで共同オーガナイズしたことも特徴であった。

現地での準備はシンガポール側で進められ、85人の参加者と35演題のポスターがあり、シンポジウム終了後にNUS-MBIの研究施設の見学ツアーを企画いただき、参加者に好評で大きな成果があったと思う。

日本側事務局には、鈴木亮先生（帝京大学薬学部・准教授）に総合事務局と、ADC登坂秘書と羽井佐事務員に事務全般に従事してもらった。そして、シンガポール側は、Prof. Paul Matsudaira, Prof. Thorsten Wohland, Prof. Linda J Kenney, Prof. Michael Sheetz and Ms. Latha Shivashankarをはじめとしたスタッフにより、スムーズに進めることができた。

詳細については、以下の記事および日本バイオイメージング学会の和文誌「バイオイメージング」を参照ください。
https://sites.google.com/site/bioimagingmag/home/new
https://sites.google.com/site/bioimagingmag/archive/080

尚、今回は、準備期間があまりなかったにもかかわらず、シンガポール側の先生方やスタッフの方々はもとより、日本側のシンポジウムのオーガナイザーおよびシンポジストの先生方、運営委員や参加者の皆様の協力により有意義なシンポジウムを開催でき感謝します。

次回の「第6回国際バイオイメージングシンポジウム」は、2019年秋、帝京大学薬学部鈴木亮先生が大会長として開催されることが学会評議委員会にて決定しており、帝京大学では、薬学部とADCで準備を開始した。

日本バイオイメージング学会
The 5th International Symposium for Bioimaging

会　期：May 20-21, 2017、都　市：Singapore
会　長：Prof. Paul Matsudaira（MBI）and Prof. Kazuo Suzuki（Bioimaging Society）
共　催：Mechanobiology Institute（MBI）
協　賛：早稲田大学WABIOS
会議場：National University of Singapore（NUS）　メイン会場＋ポスター会場
Young Scientist Travel Award：若手（40歳未満）
運　営：日本側の運営委員の太田善浩（東京農工大学大学院工学府）、岡　浩太郎（慶應義塾大学理工学部）、川西　徹（国立医薬品食品衛生研究所）、菊地和也（大阪大学大学院工学研究科）、木原　裕（姫路日ノ本短期大学）、朽津和幸（東京理科大学理工学部）、洲崎悦子（就実大学薬学部）、鈴木　亮（帝京大学薬学部）、田中直子（大妻女子大学家政学部）、橋本香保子（千葉工業大学先進工学部）、浜口幸久（東京工業大学理工学部）の各先生方

The 5th International Symposium for Bioimaging-Singapore, May 20-21, 2017 Joint Symposium on Bioimaging

Kazuo Suzuki（Director, Professor, Asia International Institute of Infectious Disease Control, Teikyo University, Japan side Chair）

Bioimaging Society held the 5th International Symposium for Bioimaging in Singapore on May 20 - 21, 2017. I served as the chair of the Japanese side together with Prof. Paul Matsudaira of the Singapore side chair. This symposium was co-hosted by Waseda Bioscience Research Institute in Singapore, WABIOS (Director: Atsushi Ishiyama). In the symposium, Prof. Shin'ichi Ishiwata (former WABIOS director) and Prof. Michael Sheetz (NUS) gave a plenary lecture. Symposium1, Symposium2

and ”Women in Science” were organized by Singapore side, Japanese side and by both side, jointly.

Singapore side made preparations on site. There were 85 attendees and 35 posters. Attendees also enjoyed the tour of NUS-MBI research facility after the symposium.

For more details, refer to the following articles and the journal: "Bio-Imaging" of Bioimaging Society.
https://sites.google.com/site/bioimagingmag/home/new
https://sites.google.com/site/bioimagingmag/archive/080

I thank Associate Prof. Ryo Suzuki (Faculty of Pharma Sciences, Teikyo University), secretary Tosaka (ADC-Teikyo University) and secretary Haisa (ADC-Teikyo University) of Japanese side and Prof. Paul Matsudaira, Prof. Thorsten Wohland, Prof. Linda J Kenney, Prof. Michael Sheetz and Ms. Latha Shivashankar of Singapore side for a great support for managing this symposium.

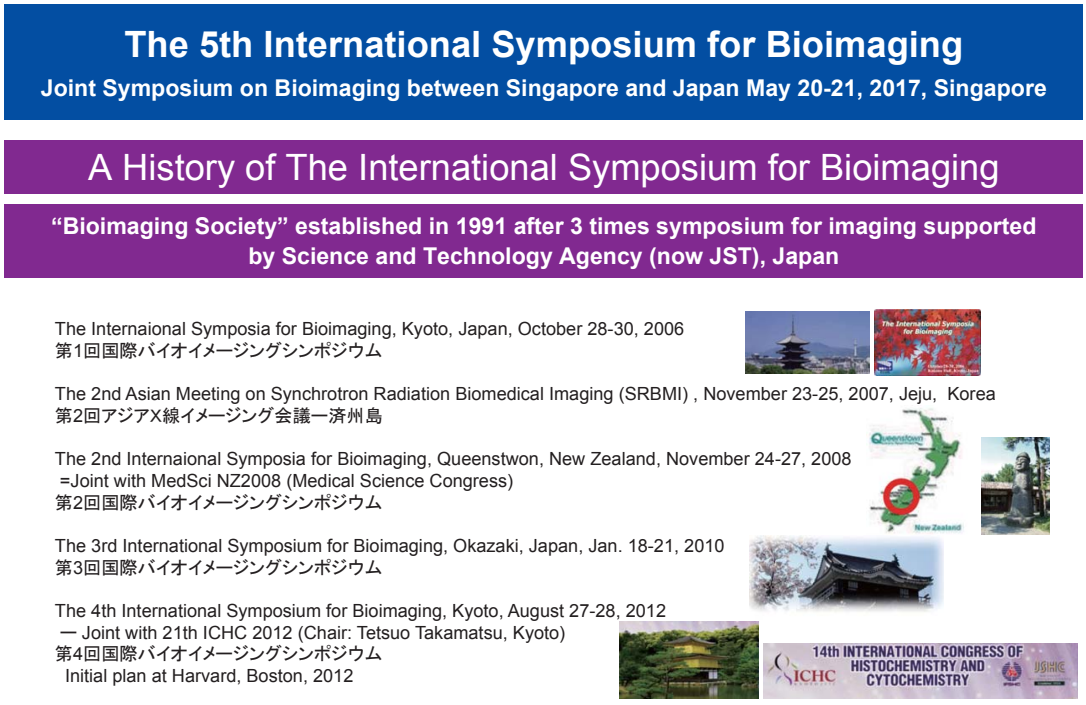
Also, I appreciate all Singapore side members as well as the organizers, symposists, committee members and attendees of Japanese side for organizing meaningful symposiums.

“The 6th International Symposium for Bioimaging” will be held by the Bioimaging Society Committee in the autumn of 2019 with the chair Associate Prof. Ryo Suzuki (Faculty of Pharma Sciences, Teikyo University). The preparation has begun with Faculty of Pharma Sciences and ADC at Teikyo University.

Bioimaging Society
The 5th International Symposium for Bioimaging

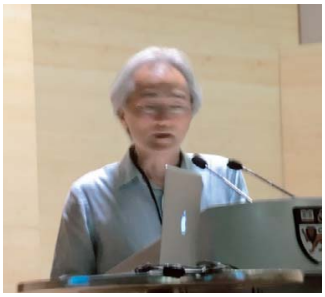
Date: May 20-21, 2017
Chairperson: Prof. Paul Matsudaira (MBI) and Prof. Kazuo Suzuki (Bioimaging Society)
Co-organized: Mechanobiology Institute (MBI)
Co-operation: Waseda University, WABIOS
Venue: National University of Singapore, Singapore
Young Scientist Travel Award: The young (under 40)
Committee member:

Yoshihiro Ohta (Institute of Engineering, Tokyo University of Agriculture and Technology), Kotaro Oka (Faculty of Science and Technology, Keio University), Toru Kawanishi (National Institute of Health Sciences), Kazuya Kikuchi (Graduate School of Engineering, Osaka University), Hiroshi Kihara (Himeji Hinomoto College), Kazuyuki Kuchitsu (Faculty of Engineering, Tokyo University of Science), Etsuko Suzaki (School of Pharmacy, Shujitsu University), Ryo Suzuki (Faculty of Pharma Sciences, Teikyo University), Naoko Tanaka (Faculty of Home Economics, Otsuma Women’s University), Kahoko Hashimoto (Faculty of Engineering, Chiba Institute of Technology), Yukihisa Hamaguchi (School of Bioscience and Biotechnology, Tokyo Institute of Technology)



Agenda

Opening Remarks



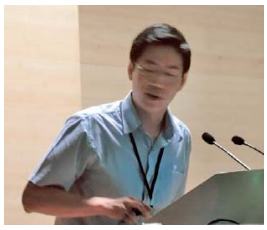
Prof. Paul Matsudaira

Symposium 1
Organizer: Prof. P. Matsudaira

Assistant Prof. Wu Min

Assistant Prof. Lu Gan

Assistant Prof. Yusuke Toyama



Assistant Prof. Pakorn (Tony) Kanchanawong

Plenary Lecture 1

Prof. Shin'ichi Ishiwata

Lunch-on Seminar JEOL

Symposium 2
Organizer: Prof. T. Nagai

Prof. Takeharu Nagai

Prof. Hiroki Ueda

Prof. Satoshi Nishimura

Associate Prof. Katsumasa Fujita

Women in Science
Organizer: Prof. L. J Kenny

Assistant Prof. Carol Tang Soo Leng, Prof. Linda J Kenney, Dr. Reina Ikaga, Associate Prof. Tomoko Masaike, Prof. N. Iida-Tanaka, Associate Prof. K. Hashimoto

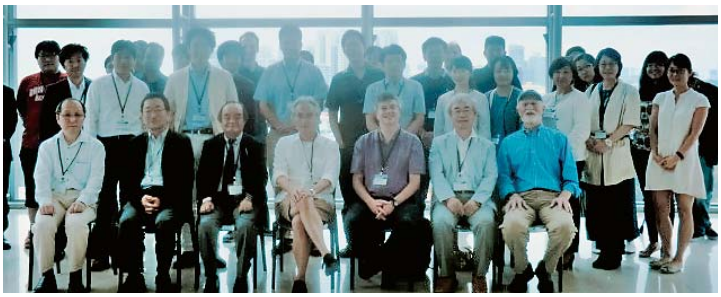
Plenary Lecture 2

Prof. Michael Sheetz

Closing Remarks

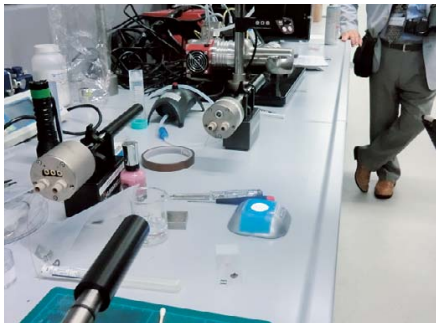
Prof. Kazuo Suzuki

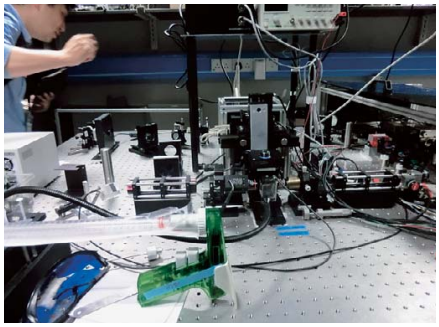
85 Attendee, 35 Posters

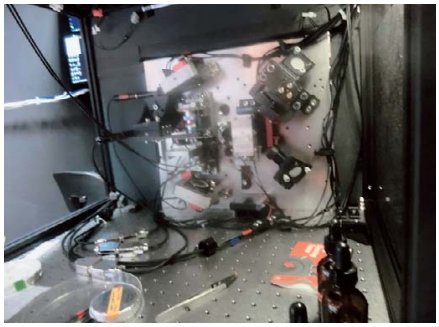


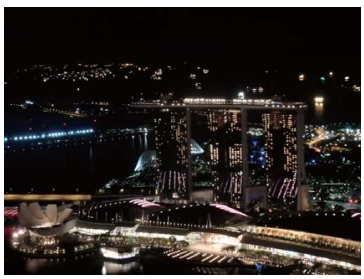
Attendee of The 5th International Symposium for Bioimaging

NUS-MBI Tour and Outing









Cytokine/chemokine changes in plasma of patients with MPO-ANCA RPGN: Before and after IVIg therapy

Kazuko Uno[1], Eri Muso[2], Toshiko Ito-Ihara[3], and Kazuo Suzuki[4]

[1]Louis Pasteur Center for Medical Research
[2]Division of Nephrology Kitano Hospital, Tazuke Kofukai Medical Research Institute
[3]Department of Clinical Innovative Medicine, Translational Research Center, Kyoto University Hospital
[4]Asia International Institute of Infectious Disease Control, Teikyo University

Corresponding author
Kazuko Uno, Ph.D.
Louis Pasteur Center for Medical Research, 103-5, Tanakamonzen-cho, Sakyo-ku, Kyoto City, Kyoto 606-8225, JAPAN
E-mail: kazukouno@louis-pasteur.or.jp

Received June 27, 2017
Accepted June 30, 2017

Abstract

Myeloperoxidase (MPO) is a subset of anti-neutrophil cytoplasmic antibodies (ANCA) and patients with this disease are highly susceptible to infection. Intravenous immunoglobulin (IVIg) therapy is considered beneficial for individuals with weak immune systems or for patients with other diseases who need to ward off infections. It has also demonstrated efficacy in suppressing disease activity in MPO-ANCA RPGN. The purpose of this study is to discuss how IVIg therapy helps to alleviate MPO-ANCA RPGN by shedding light on the mechanism of the treatment.

Cytokines/chemokines have been implicated in the pathogenesis of MPO-ANCA RPGN, therefore we determined patients' plasma cytokine/chemokine levels before and after IVIg therapy using 27 plex and 12 plex array to observe any important changes. Therapy was supplied in two dosages, full and mini dose. We observed that post-treatment levels of RANTES, IL-1α, IL-2Ra, IL-3, IL-18, CTACK, HGF, M-CSF, MIG, SCF and TNF-β decreased from their pre-treatment levels in both full dose and mini dose patients. These results suggest that these cytokines and chemokine become highly activated and are closely connected to acute MPO-ANCA nephritis, and that the efficacy of IVIg therapy is due to a reduction in these highly activated cytokine/chemokines.

Introduction

Anti-neutrophil cytoplasmic antibody (ANCA)-associated rapidly progressive glomerulonephritis (RPGN) leads to renal failure through systemic vasculitis accompanying diffuse crescentic glomerulonephritis; the representative diseases of ANCA are granulomatosis with polyangiitis (GPA) and microscopic polyangiitis (MPA). There are two known types of ANCA and proteinase-3 (PR3)-ANCA which is highly associated with GPA-ANCA and myeloperoxidase (MPO)-ANCA which is associated with MPA. This latter form is more prevalent among RPGN patients in Japan[1,2,3] and it tends to be associated with high susceptibility to infection, which frequently results in poor survival rates for these patients[4].

Ito-Ihara et al. demonstrated that IVIg therapy was effective in treating MPO-ANCA RPGN, and that in these patients, pre-treatment Plasma TNF-α levels decreased after IVIg therapy[1,5]. The objective of this study is to shed light on the characteristics and pathogenesis of MPO-ANCA and to discuss the mechanism of IVIg therapy through cytokine/chemokine fluctuations. To this end, we measured plasma cytokine/chemokine levels before and after IVIg therapy using bio-plex Group I and Group II multiplex array.

Methods

Subjects

Fifty-one patients with MPO-ANCA-RPGN were enrolled in this study. Patients were admitted to Kitano Hospital (Osaka, Japan) between January 2001 and February 2011. All patients were diagnosed with microscopic polyangitis (MPA) by the classification of Watt et al., which adheres to the definition of MPA described by the Chapel Hill Consensus Conference 2012[6]. Ethical approval was obtained from the Tazuke Kofukai Medical Research Institute Ethical Committee at Kitano Hospital.

Nineteen healthy subjects were selected from individuals who received routine health checks at Louis Pasteur Center for Medical Research (Kyoto, Japan) between January 2008 and February 2011. Healthy subjects were aged over 60, and had no history of cancer, chronic infectious diseases, autoimmune diseases, nephritis, nor asthma. Approval was obtained from the Louis Pasteur Center for Medical Research Ethical Committees (LPC.8). All participants gave written informed consent.

Treatment protocol for IVIg

Eight of the 51 MPO-ANCA -RPGN patients were treated with IVIg therapy, patient profiles are shown in Table 1. Four patients were given the full dose immunoglobulin administered intravenously once daily for 5 consecutive days (400 mg/kg/day) (Kenketus Venilon-I, Teijin Co., Ltd., Tokyo). The other four patients were treated with mini doses intravenously administered once daily for 3 consecutive days (500 mg /day). This 500 mg /day is the standard IVIg dose covered by the Japanese national health insurance for patients with severe diseases. Muso et al. separately reported clinical course and outcome which include these cases[7].

Cytokines/chemokines assay

Cytokines and chemokines were quantified using Bio-Plex 200, a multiplex cytokine array system (Bio-Rad Laboratories,

Table 1. Clinical data of patients before and after IVIg therapy

Patient No.	Age	F/M	Blood drawn interval (days)	Type of IVIg	WBC(x100/ml)		CRP(mg/dl)		Cre(mg/dl)	
					IVIg Before	IVIg After	IVIg Before	IVIg After	IVIg Before	IVIg After
1	68	M	15	400 mg/kg/day for 5 days	94	80	<0.1	0.13	2.07	2.53
2	81	F	6	400 mg/kg/day for 5 days	131	110	10.87	9.38	1.33	1.47
3	67	M	4	400 mg/kg/day for 5 days	187	137	12.84	3.47	2.77	2.13
4	88	F	5	400 mg/kg/day for 5 days	105	114	11.82	11.23	3.18	3.17
5	88	F	6	500mg/day for 3days	58	62	0.89	0.3	0.97	0.92
6	84	M	6	500mg/day for 3days	83	107	6.36	3.94	3.35	3.24
7	64	F	6	500mg/day for 3days	106	142	5.41	0.54	5.86	7.35
8	68	M	3	500mg/day for 3days	113	80	2.42	4.89	4.24	4.83

CA, USA) according to the manufacturer's instructions. Heparinized blood plasma from all 51 MPO-ANCA RPGN patients and healthy subjects were collected and centrifuged at 1,600 g for 10 min, and frozen at −80 °C until they were analyzed. We simultaneously quantified Bio-Plex Human Cytokine 27-Plex Panel and Group II -12plex array (IL-1β, IL-1Ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17, basic FGF, eotaxin, G-CSF, GM-CSF, IFN-γ, IP-10, MCP-1, MIP-1α, MIP-1β, PDGF-bb, RANTES, TNF-α, VEGF, IL-1α, IL-2Rα, IL-3, IL-12p40, IL-18, CTACK, HGF, M-CSF, MIF, MIG, SCF, TNF-β). Data acquisition and analysis were performed using Bio-Plex Manager software version 5.0.

The cytokine/chemokine values in MPO-ANCA RPGN patients and healthy controls were log-transformed and used for our analysis. All statistical analyses were carried out with JMP 9.0 software.

Results

Table 2 shows a comparison of cytokine/chemokine profiles in MPO-ANCA patients and healthy controls. Among 39 cytokine/chemokines measured, 23 were significantly higher in MPO-ANCA-patients than healthy controls.

Table 3 summarizes the clinical score and cytokine/chemokine characteristics of patients before and after full or mini dose IVIg therapy. Compared with patients treated with full dose IVIg, patients administered the mini-dose therapy showed lower cytokine/chemokine levels except for IL-12p40 and SGF (Table 3). Most cytokine/chemokine levels decreased after IVIg therapy, particularly RANTES, IL-1α, IL-2R α, IL-3, IL-18, CTACK, HGF, M-CSF, MIG, SCF and TNF-β significantly decreased in both full dose and mini dose IVIg therapy patients.

Patients treated with IVIg therapy had good prognosis and survived more than two years except one case who moved to another hospital (data not shown).

Discussion

MPO-ANCA RPGN patients are known to be highly susceptible to infection and often die due to complications arising from these infections. Ito-Ihara *et al*. have demonstrated that IVIg therapy resulted in good prognosis and low risk of renal failure[1]. In this paper, we compared MPO-ANCA patients' plasma cytokine/chemokine levels before and after IVIg therapy and compared these level with those of healthy subjects.

As shown in Table 2, pre-treatment cytokine/chemokine levels were higher in most MPO-ANCA RPGN patients than in healthy controls. Although it has been reported that several cytokine/chemokines are elevated in this disease, we believe

Table 2. Comparison of cytokine/chemokine values between MPO-ANCA patients and healthy subjects

	Healthy subjects	MPO-ANCA patients	p
log pg/ml	n=19	n=51	
IL-1b	−0.51±0.16	0.05±0.10	0.0031
IL-1ra	1.89±0.08	2.26±0.05	0.0001
IL-2	0.59±0.18	0.45±0.11	0.498
IL-4	−0.27±0.12	−0.03±0.08	0.106
IL-5	−0.37±0.14	0.41±0.09	<0.0001
IL-6	0.61±0.10	1.52±0.06	<0.0001
IL-7	−1.33±0.22	0.27±0.13	<0.0001
IL-8	1.16±0.07	1.32±0.04	0.069
IL-9	1.12±0.12	1.61±0.07	0.0008
IL-10	0.26±0.14	0.56±0.09	0.076
IL-12	−0.30±0.17	0.77±0.11	<0.0001
IL-13	−0.17±0.13	0.430±0.08	0.0003
IL-15	0.00±0.13	0.39±0.08	0.0096
IL-17	0.51±0.14	0.40±0.09	0.521
Eotaxin	1.39±0.15	1.24±0.10	0.415
FGF basic	0.95±0.19	1.06±0.11	0.605
G-CSF	−0.17±0.16	0.42±0.10	0.003
GM-CSF	1.19±0.15	1.50±0.09	0.082
IFN-g	0.50±0.22	1.22±0.14	0.0075
IP-10	2.37±0.06	2.90±0.04	<0.0001
MCP-1	1.75±0.06	1.71±0.04	0.637
MIP-1a	0.32±0.14	0.63±0.09	0.074
MIP-1b	2.00±0.53	2.03±0.03	0.659
PDGF-bb	2.19±0.13	3.00±0.08	<0.0001
RANTES	3.14±0.02	3.21±0.01	0.0024
TNF-a	0.82±0.20	0.88±0.12	0.778
VEGF	1.06±0.12	1.83±0.07	<0.0001
IL-1a	−0.50±0.13	−0.31±0.08	0.235
IL-2Ra	1.79±0.12	2.27±0.07	0.0007
IL-3	1.59±0.17	1.19±0.11	0.055
IL-12p40	0.93±0.23	1.22±0.14	0.296
IL-18	1.16±0.10	1.54±0.06	0.0012
CTACK	2.75±0.08	2.75±0.05	0.955
HGF	2.29±0.11	2.67±0.06	0.0029
M-CSF	1.32±0.09	1.79±0.06	<0.0001
MIF	1.58±0.11	2.20±0.07	<0.0001
MIG	2.68±0.09	3.51±0.06	<0.0001
SCF	2.13±0.08	2.39±0.05	0.0079
TNF-b	−0.15±0.14	0.36±0.09	0.0033

that to date this report demonstrates the most complete range of cytokine/chemokines that are elevated in MPO-ANCA RPGN.

Both full dose and mini dose therapies were effective and their outcomes were good even for patients who were over sixties. Our data demonstrated that RANTES, IL-1α, IL-2R α, IL-3, IL-18, CTACK, HGF, M-CSF, MIG, SCF and TNF-β, decreased significantly after IVIg therapy regardless of whether patients were administered a full or partial dose. Our previous study[3] demonstrated that TNF-α levels decreased significantly after IVIg therapy, and we observed the same tendency in this study. TNF-α levels decreased in three patients after IVIg therapy, two patients had levels that were unchanged and two patients showed increased TNF-α levels. A larger sample size would be ideal to fully assess the degree to which TNF-α levels actually decrease after IVIg therapy. Elevated IL-1α and IL-18 are well known to lead to cytokine storm and sepsis[8]. It is difficult to say which cytokine or chemokine is the most important to manipulate to halt the pathogenesis of MPO-ANCA

Table 3. Cytokine/chemokine values before/after IVIg therapy

	IVIg Before	IVIg After	Prob>|t|	Mini IVIg Before	Mini IVIg After	Prob>|t|	IVIgBefore vs Mini IVIg Before
log pg/ml	Mean	Mean	p	Mean	Mean	p	p
WBC(x100/μl)	129.6	110.3	NS	90.0	97.8	NS	NS
CRP(mg/l)	8.9	6.1	NS	3.8	2.4	NS	NS
Cre(mg/dl)	2.3	2.5	NS	3.6	4.1	NS	NS
IL-1b	0.68	1.01	NS	−0.19	−1.02	NS	NS
IL-1ra	2.47	2.30	NS	2.47	2.30	NS	NS
IL-2	1.04	0.95	NS	1.02	0.00	NS	NS
IL-4	0.37	0.68	NS	−0.27	−1.03	0.022	NS
IL-5	1.47	1.70	NS	0.34	−0.16	NS	0.045
IL-6	2.14	1.86	NS	1.54	0.93	0.031	0.009
IL-7	0.56	0.75	NS	0.08	−0.88	0.083	NS
IL-8	1.30	1.40	NS	1.44	1.13	0.021	NS
IL-9	1.70	1.30	NS	1.39	1.10	NS	NS
IL-10	1.30	1.26	NS	0.82	0.09	0.055	0.066
IL-12	1.17	1.30	NS	0.65	0.28	NS	NS
IL-13	0.96	1.05	NS	0.26	−0.45	NS	NS
IL-15	1.11	0.40	NS	0.94	0.00	NS	NS
IL-17	0.80	0.36	NS	0.54	0.00	NS	NS
Eotaxin	1.57	1.01	NS	0.64	0.31	NS	NS
FGF basic	1.75	1.21	NS	1.24	0.00	NS	NS
G-CSF	1.27	1.14	NS	0.39	−0.70	0.067	NS
GM-CSF	2.13	2.02	NS	1.22	0.93	NS	NS
IFN-g	1.89	2.15	NS	0.83	0.00	NS	NS
IP-10	2.70	2.79	0.012	2.97	2.88	NS	NS
MCP-1	2.00	1.91	NS	1.85	1.80	NS	NS
MIP-1a	1.37	0.93	NS	0.91	0.00	NS	NS
MIP-1b	2.10	2.06	NS	1.99	1.93	NS	NS
PDGF-bb	3.26	2.92	NS	2.63	2.95	NS	NS
RANTES	3.21	3.12	0.021	3.19	3.13	0.052	NS
TNF-a	1.78	2.04	NS	0.85	0.00	NS	NS
VEGF	2.25	1.99	NS	1.84	1.68	NS	NS
IL-1a	0.25	−0.50	0.004	−0.10	−0.69	0.035	NS
IL-2Ra	2.82	2.09	0.018	2.33	1.65	0.001	NS
IL-3	1.76	0.91	0.010	1.99	1.05	0.003	NS
IL-12p40	1.26	0.23	0.058	2.52	1.72	0.008	0.044
IL-18	1.97	1.28	0.004	1.74	1.12	0.015	NS
CTACK	2.83	2.25	0.018	3.14	2.52	0.004	0.090
HGF	3.12	2.53	0.025	3.06	2.28	0.001	NS
M-CSF	2..1593	1.31	0.002	2.06	1.08	0.002	NS
MIF	2.31	2.05	NS	2.01	1.67	NS	NS
MIG	3.59	3.05	0.001	3.70	3.14	0.002	NS
SCF	2.48	1.82	0.002	2.78	2.10	0.000	0.038
TNF-b	1.09	0.26	0.002	0.71	0.03	0.004	NS

RPGN. What we can say from the results of our study that a general decrease in elevated cytokine/chemokine tends to prevent the onset of cytokine storm.

Acknowledgments

This research was funded by Grants-in-Aid for Scientific Research, the Japan Health Foundation (No.25460784) and the Ministry of Health, Labor and Welfare of Japan (H21-S-I-004).

References

1) Ito-Ihara T, Muso E, Kobayashi S, Uno K, Tamura N, Yamanishi Y, Fukatsu A, Watts RA, Scott DG, Jayne DR, Suzuki K, Hashimoto H. 2008. A comparative study of the diagnostic accuracy of ELISA systems for the detection of anti-neutrophil cytoplasm antibodies available in Japan and Europe. Clin Exp Rheumatol 26 (6): 1027-33.
2) Watts R. A, Scott D. G, Jayne D. R, Ito-Ihara T, Muso E, Fujimoto S, Harabuchi Y, Kobayashi S, Suzuki K, Hashimoto H. Renal vasculitis in Japan and the UK - are there differences in epidemiology and clinical phenotype? Nephrol Dial Transplant 2008; 23 (12): 3928-31.
3) Ito-Ihara T, Ono T, Nogaki F, Suyama K, Tanaka M, Yonemoto S, Fukatsu A, Kita T, Suzuki K, Muso E. Clinical efficacy of intravenous immunoglobulin for patients with MPO-ANCA-associated rapidly progressive glomerulonephritis. Nephron Clin Pract. 2006; 10 2(1): c35-42.
4) Origuchi T, Ichinose K, Tamai M, Nakamura H, Kawakami A, Eguchi K. Progress in pathogenesis and therapy of vasculitis syndrome. Nihon Rinsho Meneki Gakkai Kaishi. 2007; 30 (6):432-43.
5) Taniguchi Y, Yorioka N, Yamashita K, Masaki T, Yamakido M. Expression of macrophage migration inhibitory factor in patients with myeloperoxidase anti-neutrophil cytoplasmic antibody-associated glomerulonephritis. Clin Nephrol. 2000; 54(2): 147-50.
6) Jennette J. C, Falk RJ, Bacon PA, Basu N, Cid MC, Ferrario F, Flores-Suarez LF, Gross WL, Guillevin L, Hagen EC, Hoffman GS, Jayne DR, Kallenberg CG, Lamprecht P, Langford CA, Luqmani R. A, Mahr A. D, Matteson E. L, Merkel P. A, Ozen S, Pusey CD, Rasmussen N, Rees AJ, Scott DG, Specks U, Stone JH, Takahashi K, Watts RA. 2013. 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. Arthritis Rheum; 65(1):1-11.
7) Muso E, Ito-Ihara T, Ono T, Imai E, Yamagata K, Akamatsu A, Suzuki K. Intravenous Immunoglobulin(IVIg) Therapy in MPO-ANCA Related Polyangitis with Rapidly Progressive Glomerulonephritis in Japan. Jpn. J. Infect. Dis. 2004; 57: S17-8.
8) Chaudhry H, Zhou J, Zhong Y, Ali M. M, Mcguire F, Nagarkatti P. S, Nagarkatti M. Role of Cytokines as a Double-edged Sword in Sepsis. In Vivo. 2013; 27(6): 669-84.

ORIGINAL ARTICLE

Establishment of a library having 204 effective clones of recombinant single chain fragment of variable region (hScFv) of IgG for vasculitis treatment

Yosuke Kameoka[1*], Minako Koura[2], Junichiro Matsuda[2], Osamu Suzuki[2], Naohito Ohno[3], Ryuichi Sugamata[4#], Toshinori Nakayama[4], and Kazuo Suzuki[4#]

[1]National Institutes of Biomedical Innovation, Health and Nutrition, Laboratory of Rare Disease Biospecimen, Saitoasagi 7-6-8, Ibaraki-Shi, 567-0085, Osaka, Japan
[2]National Institutes of Biomedical Innovation, Health and Nutrition, Laboratory of Animal Models for Human Diseases, Saitoasagi 7-6-8, Ibaraki-Shi, 567-0085, Osaka, Japan
[3]Laboratory for Immunopharmacology of Microbial Products, School of Pharmacy, Tokyo University of Pharmacy & Life Sciences, 1432-1 Horinouchi, Hachioji, Tokyo, 192-0392, Japan
[4]Department of Immunology, Graduate School of Medicine, Chiba University, Inohana 1-8-1, Chuo-ku, Chiba-City, Chiba, 260-8640, Japan

*Present address: A-CLIP Institute, Ltd. Inohana 1-8-15, Chuo-ku, Chiba-City, Chiba, 260-0856, Japan
#Present address: Asia International Institute of Infectious Disease Control, Teikyo University, Kaga 2-11-1 Itabashi-ku, Tokyo, 173-8605, Japan

Correspondence
Yosuke Kameoka: A-CLIP Institute, Ltd. Inohana 1-8-15, Chuo-ku, Chiba-City, Chiba, 260-0856, Japan

Received June 28, 2017
Accepted June 30, 2017

Abstract

Purpose Vasculitis is a refractory disease with no established treatment. Therapeutic effect of gamma globulin (IgG) in high dose therapy (IVIg) has been reported[1]. IgG preparation which depends on human-derived material, is not completely safety and supply amount of it is finite. Development of recombinant gamma globulin having the same effect has been requested.

Methods We constructed a library of recombinant single chain fragment of variable region (hScFv) of IgG from the constitution of VH-CH1-hinge from peripheral blood lymphocytes of healthy volunteers. One thousand recombinant hScFv clones were analyzed in base sequence, and selected by having the correct structure of hScFv. Selected clones were mixed and cultured, and induced hScFv proteins were administered to spontaneous vasculitis mouse model SCG/Kj to evaluate therapeutic effects.

Results From the base sequence analysis of 1,000 clones, we obtained 245 clones having VH-CH1-hinge structure, then a library having 204 clones was established. All 204 clones were mixed and cultured to induce hScFv proteins. A purified preparation was administered to SCG/Kj mice. The hScFv administration at a concentration of 1/20 of the large amount of IgGs preparation showed same effect for suppression of glomerular crescent formation and a refinement of the peripheral blood cell was observed in hScFv administration groups. Also, a biomarker MPO-ANCA titer remarkably decreased administration dose of IVIg in 1/10

Conclusions The hScFv protein mixture showed therapeutic effects with 1/10-1/40 of IVIg in administration to SCG/Kj mouse. It seems to be a development possibility to actual preparation as recombinant IgG.

Key words: Single chain antibody fragment of variable region (hScFv), Immunoglobulin preparation, Vasculitis, antibody drug, spontaneous vasculitis mouse model SCG/Kj

Introduction

Immunoglobulin preparation therapy is an effective treatment for patients with vasculitis[1]. Microscopic polyangiitis and anti-nuclear cytoplasmic antibody (ANCA)-associated vasculitis (AAV)[2], are an intractable vasculitis. These have been frequently seen in Japanese people as approximately 10,000 registered cases in medical expense support by the Japanese government, especially in seniors[3]. Its underlying lesion is a systemic inflammation of small vessels. A high percentage of patients have auto-antibody against myeloperoxidase (MPO-ANCA). Most of patients with rapidly progressive glomerulonephritis have MPO-ANCA-associated vasculitis (MAAV), alveolar hemorrhage, and interstitial pneumonia with a poor prognosis. Although immunosuppressive agents including high dose steroids are used for AAV treatments, some patients require dialysis because of a rapid decline of renal function. Other vasculitis-related diseases are children's vasculitis Kawasaki disease[4], which are treated with immunoglobulin preparation (IVIg). Also, IVIg treatement has been used for infectious diseases[5], and has been applied to MAAV[6]. However, IVIg treatment requires high cost and short supply and has risk of unknown causes of infection due to human blood preparations. Therefore, pediatricians and patient's parents are expecting the advent of recombinant IgGs as therapeutic drugs to evade such risks.

We established a recombinant human single-chain IgG library consisting of 204 clones as a therapeutic drug for intractable vasculitis. The hScFv library was established from peripheral blood mono-nuclear cells of the several ten healthy donors. Therapeutic effect of the combined recombinant hScFv clones from the library is evaluated by using a spontaneous vasculitis mouse model SCG/Kj[7].

Methods

hScFv cDNA library construction

Total RNA including IgG coding mRNA was isolated from the mononuclear cells in the peripheral blood from healthy

donors under approval of the ethical committee in National Institute of Biomedical Innovation, Health and Nutrition Japan (No. 51). The cDNA fragment coding VH-CH1-hinge region of IgG was obtained by using the reverse transcription PCR with the primer set described in Figure 1. The cDNA fragment was inserted into the correct position, which is in front of the hexahistidine tag of the plasmid vector pBAD[8]. The hScFv fragment inserted plasmid vector was chemically transfected to Top10 competent cells (Life Technologies, USA). The hScFv including recombinant library was consisted of 20,000 clones (Figure 1).

Base sequence analysis and cluster analysis of hScFv clones

One thousand recombinant clones were picked up in random from the constructed library. Inserted fragments of each clones were amplified by pBAD primer set, as described in Figure1, then the base sequence of amplified inserted fragments of each clone was analyzed by 3730 DNA analyzer (ABI, USA). Base sequence analysis of inserted fragments was carried out in both direction, forward and reverse read. The base sequence data was analyzed and edited certainly by Factura software (ABI, USA).

Deduced amino acid sequence of each clone was obtained by base sequence results. The amino acid sequence of each hScFv clone was carried out by cluster analysis with ClustalW (open source, URL http://www.clustal.org/clustal2/) software to confirm the difference of each clone[9, 10].

Purification of hScFv protein from the mixed culture cells

Ten ml of mixed clone precultured cells were inoculated to 1L of LB broth supplemented with 50 micro g of Ampiciline. Mixed culture was incubated at 37°C with shaking until OD600=0.6, then arabinose was added to 0.05% to induce hScFv protein expression. After addition of arabinose, culture was continued for 16hr. Cultured cells were collected by centrifugation, and separate cell pellet. The cell pellet was freeze-thawed three times, and suspended in 20 ml of extraction buffer (8 M urea, 10 mMTris pH 7.0), then the cells were disrupted by sonication with 80% output power of VP-050 (TITEC Gunma, Japan). The soluble fraction was separated by centrifuge at 5,000 rpm for 15 min. Soluble fraction was applied to Ni-NTA column including 1ml of resine. The column was

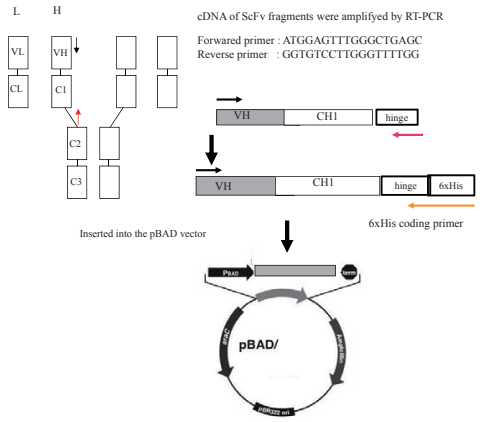


Figure 1. Construction of hScFv library
The cDNA coding VH-CH1- hinge region was amplified by RT-PCR, using primer set described in the figure. Amplified cDNA was attached to hexahistidine tag by primer extension. The fragments were cloned into the pBAD vector, which restricted transcription strictly.

washed with 10 ml of extraction buffer, then washed with extraction buffer supplemented with 5 mM glycine. The resin binding proteins was eluted with 5 ml of elution buffer (8 M urea, 10 mM TRIS pH 7.0, 0.1 M EDTA). Purified protein was examined by SDS-PAGE and western blot to confirm the purity, property and amount[11].

SDS-PAGE and western blot analysis of each clone

hScFv proteins-expressed clones were selected. hScFv expressed clones were cultured individually in mini scale (5 ml culture each), and they induced hScFv protein expression by adding 0.05% arabinose for 16 hrs. 20 micro L of cultured cells were collected by centrifuge, and then the cells were re-suspended in SDS sample buffer (0.05% SDS, 10 mM Tris pH 6.8, 2 mM DTT) and separated on the 4-20% polyacrylamide gel. The separated proteins were transferred to the PVDF membrane electrically, and hScFv on the PVDF membrane was detected by alkaline phosphatase conjugated anti-human Fab2 antibody (Rockland, USA). Isolated hScFv proteins were also applied with the same procedures.

Administration of hScFv for spontaneously vasculitis mouse model SCG/Kj

Mice care was approved by the committee in the National Institutes of Biomedical Innovation, Health and Nutrition (No. DS21-8).

Administration program was established as well as clinical administration of IVIg[4]. The administration was performed with 10-week old female spontaneous vasculitis mouse model SCG/Kj intraperitoneally at doses of 0, 10, 20, and 40 mg/Kg/day of hScFv for 5 days continuously. As a control, according to the application amount of doses, human globulin preparation (Nihon Pharmacy Company, Osaka, Japan) was administered with 400 mg/kg/day for 5 days continuously.

Administration dose was set in five groups, 1) 0 mg/kg/day (solvent), 2) 10 mg/kg/day, 3) 20 mg/kg/day, 4) 40 mg/kg/day, 5) 400 mg/kg/day which was the same dose to IVIg. 10-week old female SCG/Kj mice were administered for 5 days continuously with same dose for each group.

Evaluation of hScFv after administration into SCG/Kj mice

After observation for 3 weeks since administration, blood, kidney, lung, heart, spleen were sampled and weighed. Blood component analysis was performed with blooding and scarified under anesthesia, and excised spleens were weighted. Peripheral blood was examined by a VetScan HMII (Abaxis, USA) to analyze peripheral blood cell counts. Kidney was fixed with 10% formalin and embedded in paraffin block and stained with Hematoxilin and Eosin, then approximately 50 of glomerular crescents in the kidney were counted under a microscope. MPO-ANCA was performed with ELISA methods.

Results

hScFv of VH-CH1-hinge fragment, originated from healthy donor peripheral blood lymphocytes and inserted in the expression plasmid pBAD library, was constructed. One thousand clones were picked up in random. The clones were analyzed for base sequence of inserted VH-CH1-hinge coding region. 245 clones in one thousand could express the protein correctly jointed to histidine tag. 224 clones in 245 were

certainly coding the VH-CH1-hinge. 46 clones were redundant clones. Finally, 204 clones coding unique VH-CH1-hinge-6xHis structure were obtained.

Cluster analysis of deduced amino acid sequence of hScFv

The deduced amino acid sequence of hScFv clones were analyzed by using the clustal W software. 21 clones revealed broken structure of hScFv. Other 223 clones were ordered by resemblance of the sequence, departed in several groups (Figure 2).

Confirm the Expression of hScFv each clone by SDS-PAGE and western blot

hScFv expressed selected clones were cultured individually and they induced hScFv protein expression. After SDS-PAGE separation of each clone, we examined them by western blot with alkaline phosphatase conjugated anti-human IgG and anti-human Fab2 antibody. Each clone was confirmed as a human IgG molecule and Fab2 fragment in correct size of 28 kDa (data not shown).

hScFv protein extraction and purification

Expressed protein in *E. coli* cells from total 30 L culture was extracted by 8 M urea, and purified by Ni-NTA chromatography. Obtained hScFv protein showed over 90% purity by SDS-PAGE analysis, confirmed by western blot analysis by using anti-human IgG Fab2 antibody. Finally, 3 mg of hScFv mixed protein were recovered from 30 L culture. Obtained hScFv eliminated endotoxin by alkaline hydrolysis in the level of under 10 unit/ml.

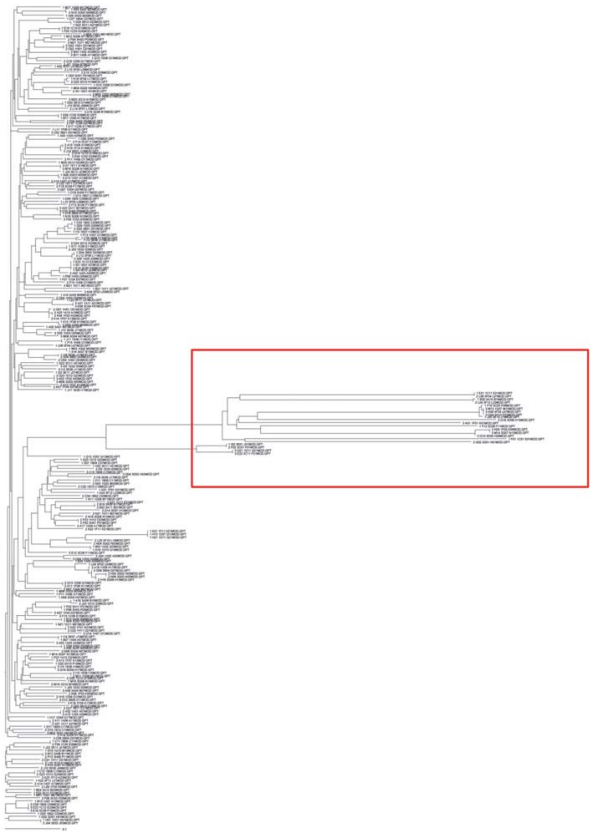


Figure 2. Cluster analysis of hScFv clones
Difference of amino acid sequence of each hScFv clone was analyzed by using Clustal W software. hScFv clones made several clusters by resemblance of amino acid sequence of VH region. The clones with broken structure made separate group in a rectangular of red line.

Final solution of hScFv protein was solved in 0.9 M Arginine, 0.9 %NaCl pH7.4 (Figure 3).

Evaluation of hScFv after administration into vasculitis mouse model SCG/Kj

The glomerular crescent formation in histological analysis of kidney showed significant decrease in the dose of 20 mg/kg/day administration compared to that of control (solvent administration) (Figure 4A). MPO-ANCA titer, a biomarker of vasculitis, which is auto-antibody against to myeloperoxidase showed also significant decrease at the administration dose of 40 mg/kg/day compared to that of control group (Figure 4B). The number of whole blood cell indicated significant decrease

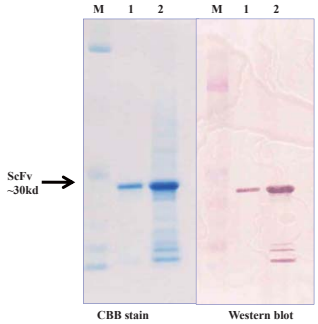


Figure 3. Purified hScFv mix clone proteins
The protein which was purified from hScFv mixed culture were examined by SDS-PAGE and stained by CBB. The separated proteins were blotted to PVDF membrane and examined by immunological detection, using anti-human IgG Fab2 antibody. Lane M: molecular marker, 1:0.1 micro g purified hScFv applied, 2: 1 micro g of hScFv applied.

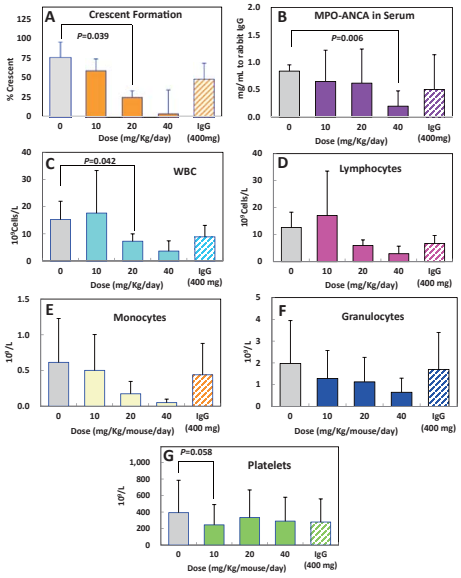


Figure 4. Evaluation of hScFv treatment with kidney histology and biomarkers
A: Crescent formation rate in kidney (orange color bar), The groups of 20 and 40 mg/kg/day administration indicated more decrease of spleen weight. B: MPO-ANCA titer (violet color bar) MPO-ANCA titer was decreased in dose dependent manner. The titer of administration dose 40 mg/kg/day showed remarkable decrease than that of IvIG administration. C: Counts of white blood cells in peripheral blood; whole blood cells (WBC: light blue color bar), D: lymphocytes (red color bar), D: monocytes (yellow color bar), E: granulocytes (dark blue color bar), F: platelets (green color bar). Controls treatment with solvent (gray color bar) and IgG 400 mg/Kg/day for 5 days (hatched bar) in each data. Statistical analysis was performed between solvent control vs treatment group by Student's *t*-test (p-value).

at the dose of 20 mg/kg/day compared to that of control (Figure 4C). The number of lymphocytes, monocytes, granurocytes and platelet in peripheral blood decreased slightly in hScFv administration group, however the deference was not significant (Figure 4 D, E, F, G).

Discussion

A recombinant human ScFv library consisting 204 clones of VH-CH1-hing was established from a 1,000 recombinant clone library. Purified hScFv was recovered from a mixed batch culture of polyclones, and 0.1 to 0.2 mg per 1 L of the final purified preparation was realized, because the production level in *E. coli* was quite low.

The purified hScFv was administered to SCG/Kj mice for examination of the therapeutic effect. After three week observation, the therapeutic results were observed in decreasing the number of the glomerular crescent formation and peripheral whole blood cell in hScFv administered groups. It has obtained comparable results at concentrations of 1/10 to 1/40 of the IgG preparations which are currently used clinically.

It has been showed the possibility for actual formulation as a recombinant gamma globulin. In addition, the solubility of the VH-CH1-hinge complex was considered to be increased to use as a therapeutic drug.

It was possible to obtain an effective soluble preparation at low concentration compared to the gamma globulin preparation. It seems to be hopeful results that we approach the actual drug preparation. Antibody drug development becomes important for the therapeutic strategy in the field of various diseases, which depend on the new resources for new antibody drugs[12]. This study gives a useful new resource for the development of next generation antibody drug.

By collecting effective clones from larger libraries, it is possible to create recombinant gamma globulin with higher effect. These 204 clones library will be useful for infectious diseases.

Conclusion

The administration of the mixed batch of recombinant gamma globulin hScFv 204 clones having the VH-CH1-hinge composition showed inflammation inhibitory activity such as MPO-ANCA.

Acknowledgements

This work was supported in part by grants-in aid for the Gamma globulin Project from the Ministry of Health, Welfare and Labour. We thank Drs. Kikuko Takeuchi, Masao Takeuchi and Mr. Makoto Hirata and Ms. Keiko Uchida of National Institutes of Biomedical Innovation, Health and Nutrition, Laboratory of Rare Disease Biospecimen for great support for managing of this study.

Reference

1) Ito-Ihara T, Ono T, Nogak F, Suyama K, Tanaka H, Yonemoto S, Fukatsu A, Kita T, Suzuki K, Muso E. Clinical efficacy of intravenous immunoglobulin for patients with MPO-ANCA-associated rapidly progressive glomerulonephritis. Nephron Clinical Practice 2005; 102: C35-42.
2) Jennette JC, Falk RJ, Bacon PA, Basu N, Cid MC, Ferrario F, Flores-Suarez LF, Gross WL, Guillevin L, Hagen EC, Hoffman GS, Jayne DR, Kallenberg CG, Lamprecht P, Langford CA, Luqmani RA, Mahr AD, Matteson EL, Merkel PA, Ozen S, Pusey CD, Rasmussen N, Rees AJ, Scott DG, Specks U, Stone JH, Takahashi K, Watts RA. 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. Arthritis & Rheumatology 2013; 65(1): 1-11.
3) Kobayashi S, Fujimoto S, Takahashi K, Suzuki K. Anti-neutrophil cytoplasmic antibody-associated vasculitis, large vessel vasculitis and kawasaki disease in Japan. Kidney and Blood Pressure Research 2010; 33(6): 442-55.
4) Kobayashi T, Saji T, Otani T, Takeuchi K, Nakamura T, Arakawa H, Kato T, Hara T, Hamaoka K, Ogawa S, Miura M, Nomura Y, Fuse S, Ichida F, Seki M, Fukazawa R, Ogawa C, Furuno K, Tokunaga H, Takatsuki S, Hara S, Morikawa A; RAISE study group investigators.Efficacy of immunoglobulin plus prednisolone for prevention of coronary artery abnormalities in severe Kawasaki disease (RAISE study): a randomised, open-label, blinded-endpoints trial. Lancet. 2012; 28: 379(9826): 1613-20. doi: 10.1016/S0140-6736(11) 61930- 2.
5) Farag N, Mahran L, Abou-Aisha K, El-Azizi M. Assessment of the efficacy of polyclonal intravenous immunoglobulin G (IVIG) against the infectivity of clinical isolates of methicillin-resistant Staphylococcus aureus (MRSA) in vitro and in vivo. Eur J Clin Microbiol Infect Dis. 2013; 32(9): 1149-60. doi: 10.1007/s10096-013-1861-5.
6) Muso E, Ito-Ihara T, Ono T, Imai E, Yamagata K, Akamatsu A, Suzuki K. Intravenous immunoglobulin (IVIg) therapy in MPO-ANCA related polyangiitis with rapidly progressive glomerulonephritis in Japan. Jpn J Infect Dis. 2004; 57(5): S17-8.
7) Tomizawa K, Nagao T, Kusunoki R, Saiga K, Oshima M, Kobayashi K, Nakayama T, Tanokura M, Suzuki K. Reduction of MPO-ANCA epitopes in SCG/Kj mice by 15-deoxyspergualin treatment restricted by IgG2b associated with crescentic glomerulonephritis. Rheumatology. 2010; 49 (7): 1245-56.
8) Guzman LM, Belin D, Carson MJ, Beckwith J. Tight regulation, modulation, and high-level expression by vectors containing the arabinose PBAD promoter. J Bacteriol. 1995; 177(14): 4121-30.
9) Guex, N., Peitsch, M. C., & Schwede, T. Automated comparative protein structure modeling with SWISS-MODEL and Swiss - PdbViewer: A historical perspective. *Electrophoresis*, 2009; 30(S1), S162-73.
10) Pansri, P., Jaruseranee, N., Rangnoi, K., Kristensen, P., & Yamabhai, M. A compact phage display human scFv library for selection of antibodies to a wide variety of antigens. BMC biotechnology 2009; 9(1), 6.
11) Chen LH, Huang Q, Wan L, Zeng LY, Li SF, Li YP, Lu XF, Cheng JQ. Expression, purification, and in vitro refolding of a humanized single-chain Fv antibody against human CTLA4 (CD152). Protein Expr Purif. 2006; 46(2): 495-502.
12) Beck A, Goetsch L, Dumontet C, Corvaïa N. Strategies and challenges for the next generation of antibody-drug conjugates. Nat Rev Drug Discov. 2017; 16(5): 315-37. doi: 10.1038/nrd.2016.268.

TAVP TRAINING FOR STUDENTS

医学部5年生：公衆衛生学実習「19：ベトナム感染症」

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て学習

研修する科：

・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：感染症疫学

8月20日（日）朝、成田出発
8月27日（日）早朝、成田着（26日夜発）
付き添い：鈴木和男、河内正治、医学部教員

（応募時メンバー）Kazuo Suzuki, Hinako Takamura, Chisato Urui, Aya Nagasaka, Naohiro Ichikawa, Masayuki Sone, Takayoshi Okubo
鈴木和男、髙村日菜子、閏井智郷、長坂　彩、市川尚寛、曽根雅之、大久保孝義
Chisa Narikawa, Minori Hata, Yasuko Horie, Yuuki Mitani, Sakiko Asano
成川智彩、秦　美能理、堀江恭子、三谷優季、朝野紗稀子

STEERING COMMITTEE RECORD

2016年度　ADC運営委員会記録

日　時：2017年3月16日（木）16時00分～18時00分　場　所：帝京大学本部棟4F
議事・討議・審議内容

1．病院長あいさつ　帝京大学医学部附属病院病院長　坂本哲也
ADC研の成り立ちの原点は、*Acinetobacter*による感染であった。それにより感染症の安全管理に力を入れてきた。また、アジアからの留学生を受け入れられてうれしく思う。

2．平成28年度事業報告
1）プログレスレポート
①鈴木和男　②菅文龍一、Tran Huu Dat　③鈴木章一、Nguyen Thu Thuy　④伊藤吹夕
2）プロジェクト研究
e-ASIAの最終年度に当たり、28年度最終全体（3か国）会議は Philippines Manilaで行った。かねてよりPhilippinesから提案されていた Leukomycin A_3（Josamycin）の臨床試験が開始された。
ベトナム保健省 People's Health メダル受賞：鈴木和男、河内正治
ベトナム国立小児病院客員教授：鈴木和男
3）国際交流
①国立小児病院（ハノイ）およびハノイ医科大学で単位互換に基づき医学部5年生の7名の学生実習
②帝京大学創立50周年ADC国際シンポジウム（臨床大講堂）

3．平成28年度事業報告の承認：出席20名、委任状11名（運営委員数37名）

4．平成29年度事業計画案
1）e-ASIA：ベトナムでは百日咳を中心に、フィリピンは結核を中心として計画
2）帝京大学アジア研修プログラムとして、医学部5年生10人がベトナムで研修・実習をする。
3）ADC Letter が4月からJ-STAGEに掲載される。

5．平成29年度事業計画案が承認された。出席20名、委任状11名（運営委員数37名）

6．あいさつ
滝川　一（医学部長）、栗原順一（薬学部長）、山岡和枝（公衆衛生学研究科教授）

INTERNATIONAL MEETING AND SYMPOSIUM

開催したイベント　（2017.1.1~2017.6.30）

日程	イベント名	演者など	
2017年5月9日(火)	危機管理と防災	板橋キャンパス危機管理委員会、ADC	臨床大講堂
2017年3月12日(日)~3月14日(火)	e-ASIA Project Meeting	Josamycin Transport	Manila, Philippines
2017年3月9日(木)	バイオセーフティ講習会	ADC	セミナー室
2017年2月19日(日)~2月22日(水)	e-ASIA Project Meeting	Discussion of Project Progress Report of 2016 FY(Flu Team)	Hanoi, Vietnam
2017年1月22日(日)~1月23日(月)	e-ASIA Project Meeting	LM-A3 Protocol Preparation	Manila, Philippines

今後のイベント情報　（2017.7.1~2017.12.31）

日程	イベント名	演者など	
2017年12月1日(金)~12月2日(土)	第23回　MPO研究会	ADC	帝京大学　板橋キャンパス
2017年11月頃	14th　International Symposium	金谷泰宏	臨床大講堂
2017年10月15日(日)~10月24日(火)	SAKURA Science Plan 2017	Vietnamから6名の研修生	
2017年8月20日(日)~8月27日(日)	TASP Training for 10 Students (5-year)	NHP and Bacmai Hospital	Hanoi, Vietnam