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ADC Letter
for Infectious Disease Control

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TEIKYO


Histology of the vascular lesions (HE stain, ×40)

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

EDITORIAL

6巻2号をお届けします。
毎回、新しいプログラムとプロジェクトが始まり、多様になってきています。
東南アジアとともに、米国、欧州の研究者らとの交流もさらに活発になりました。
本号記載のADCの事業は、以下になります。

【研究プロジェクト、感染症制御研究】

1）研究ブランディング事業：パンデミック感染症の病原体に関するベトナムとの共同研究を進めています。
2）医学研究科大学院：大学院生4年生のTran Huu Dat君は、The Journal of Antibioticsに論文受理、特許申請が終わり、医学博士学位申請中です。
3）ベトナムでも川崎病が日本と同様の頻度で確定診断されはじめました。

【アジア諸国および欧米の医療機関との研究交流】

1）2019年度の医学部5年生「ベトナム感染症実習」（4期生）6名が準備中
　期　間：2019年8月18日（日）～25日（日）
　研修先：国立小児病院、ハノイ医科大学、Vinmec国際病院、ハノイ郊外の保健所など
2）医学部6年生の海外BSC
　2019年度（2期生）：笠井健司君（米国ボストン）、長久大介君（英国ケンブリッジ）
　2020年度（3期生）公募中、審査上位3名に奨学金
3）「さくらサイエンスプラン」4期生
　10月28日（月）～11月6日（水）の10日間
　研修目的：「安全管理」、「感染制御」、「危機管理」、「シミュレーション」など

【Stem Cell Therapy Consortium：SCTC】

ADC内にSCTCブランチとして設置準備
基礎研究を進め、臨床試験を準備中で、先端総合研究機関とも連携を予定
・米国NIH/NICHD Dr. Keiko Ozatoおよびベトナム Vinmec国際病院附属研究所長（元、国立小児病院長）との共同研究

We are pleased to issue ADC letter Volume 6 No. 2.
Some new programs and projects have been started and various studies are active.
Particularly, communication with investigators in not only Southeast Asia countries, but the United States, and European countries have been active too.
The studies in the ADC include the followings:

【Research projects: infectious disease control study】

1) Research of Blanding: Study on the pathogens of the pandemic infectious diseases to be able to do gene analysis through collaborative investigation with Vietnam is in progress.
2) Medical graduate student fourth grader, Dr. Tran Huu Dat: Acceptance of his research article in The Journal of Antibiotics, and patent application were completed, then he applied his thesis for the graduate student.
3) Frequency of Kawasaki disease diagnosis in Vietnam is similar as Japan.

【Exchange program with medical institution in the Asian countries and US-European countries】

1) Six fifth-grade medical students will join "Vietnam infectious disease training 2019: 4th period" and 6 students will visit National Children's Hospital (NCH), Hanoi Medical University, the public health center of the Hanoi suburbs from August 18 to 25th, 2019.
2) Overseas BSC of the sixth-grade medical students
　2019 (2nd period): Kenji Kasai (Boston, USA), Daisuke Chokyu (Cambridge, UK)
　2020 (3rd period): Scholarship: Open for all 6 grade students, and then three students ranked highest will be accepted.
3) Training program in Teikyo University "Sakura Science Plan 2019: 4th period" from Monday, October 28 to Wednesday, November 6: about "Safety Management", "Infection Control", "Crisis Control", "Simulation".

【Stem Cell Therapy Consortium】

SCTC Branch in ADC will be organized.
A plan for basic sciences has been prepared followed by a clinical trial associated with the "Advanced Research General Institute Organization". This project plan has been collaborated with Dr. Keiko Ozato in NIH/NICHD, USA and Prof. Nguyen Liem in the Vietnamese Vinmec Research Institute of Stem Cell and Gene Technology, Hanoi.

編集長：鈴木和男 Editor-in-Chief : Kazuo Suzuki, Director　　**事務局：伊藤吹夕** Editorial Office : Fuyu Ito, Ph.D.

表紙写真：*Candida albicans* water-soluble fraction（CAWS）誘導マウス血管炎。川崎病血管炎に類似する冠状動脈炎が惹起される。マウスはC57BL/6、HE染色、x40。大原関利章先生（東邦大大橋病院）ご提供。
Histology of Coronary Arteritis in Murine Vasculitis Induced by *Candida albicans* Water-Soluble fraction (CAWS). (Mouse C57BL/6, HE stain, x40) By Dr. Toshiaki Oharaseki, Toho Univ. Ohashi Medical Center.

The 19th International Vasculitis and ANCA Workshop

April 7-10, 2019

2年に1回開催されている国際血管炎ANCA Workshopは、今回ペンシルバニア大学のPeter Merkel教授（写真中央）が会長となり2019年4月7日～10日、フィラデルフィアにて開催されました。

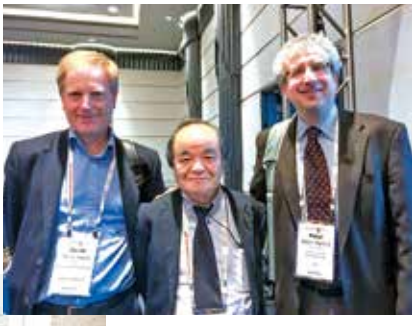
プログラムの概略は下のようでした。圧巻だったのは、招待講演のDr. David Fajgenbaum（写真右）の講演でした。医師である本人が、キャッスルマン病と診断されるまでの苦闘と、アクテムラ（IL-6R抗体医薬）の治療に出会いそれによって回復した話をムービーを交えて話してくれました。現在は、ペンシルバニア大学で医師として働いているという話に会場にいた参加者は総立ちのオベーションでした。また日本からも多くのプレゼンがあり反響も大きかったです。次会は、2021年春アイルランド・ダブリンのMark Little教授が決まっています。

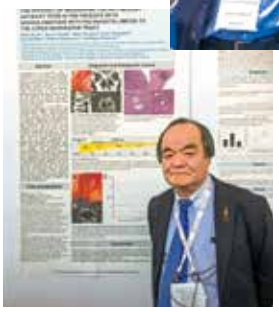
【The 19th International Vasculitis meeting ANCA Workshop】

The International vasculitis meeting and ANCA Workshop has been held once in two years. This time it was held in Philadelphia from 7 to 10 April in 2019. Prof. Peter Merkel (center of a photography) of Pennsylvania University was a Chairperson in this meeting. The outline of the program is shown in the list. The invitation lecture by Dr. David Fajgenbaum (rightside of a photography) was the best part in the meeting. While he was a doctor, he talked about the story that he contacted Castleman disease and hard fight before having a diagnosis and Actemura (antibody to IL-6R) treatment, and recovered by the treatment as shown in a movie. After recovery, he works as a doctor in Pennsylvania University. All audiences were cheered by his talk and he had standing up obation. There were some presentations from Japanese researchers, and had a good reputation. The next meeting will be held in Dublin, Ireland with a chairperson Prof. Mark Little in the spring of 2021.

AGENDA AT-A-GLANCE

Sunday, April 7, 2019	
3:00-3:30	**WELCOME** [Regency Ballroom] Workshop Chair: Peter Merkel
3:30-4:30	**INTRODUCTORY REMARKS** [Regency Ballroom] Francis F. Collins, MD, PhD **FOKKO VAN DER WOUDE LECTURE S1** Carl June, MD "CAR-T Cell Therapy in Autoimmune Disease"
Monday, April 8, 2019	
9:30-10:20	**INVITED SPEAKER** [Regency Ballroom] David Fajgenbaum, MD, MBA, MSc "Personalized Medicine"
Tuesday, April 9, 2019	
9:30-10:20	**INVITED SPEAKER** [Regency Ballroom] Rae S. M. Yeung MD, PhD, FRCPC "Towards Precision Medicine-Lessons Learned from Kawasaki Disease"
Wednesday, April 10, 2019	
12:20-12:30	**CLOSING REMARKS/WRAP-UP** [Regency Ballroom]





EUVAS-VCRCIM in Philadelphia, USA

April 7, 2019

「European vasculitis society/vasculitis clinical research consortium investigators meeting」が、プログラムにあるように、2019年４月７日（日）9：00 ～ 14：00にLoews Hotel, Philadelphia USAで開催されました。例年通り、オーガナイザーは、PETER A. MERKEL（ペンシルバニア大学教授）とDAVID JAYNE（ケンブリッジ大学教授）で、日本から２つの提案をしました。１つは、東北医薬大学の川上民裕皮膚科教授のARAMISの日本事務局からの状況報告（猪原登志子先生：京都府立医大が代行）、もう１つは東邦大学医学部本間栄教授による肺限局型血管炎の日本の状況報告（鈴木和男教授：帝京大学が代行）でした。

EUROPEAN VASCULITIS SOCIETY / VASCULITIS CLINICAL RESEARCH CONSORTIUM

VASCULITIS INVESTIGATORS MEETING

Sunday April 7, 2019: 9:00 AM – 2:00 PM

Loews Hotel, Philadelphia USA

CHAIRS: PETER A. MERKEL & DAVID JAYNE

WELCOME AND INTRODUCTIONS

1) CLINICAL EPIDEMIOLOGY & GENETICS STUDIES ..... C. Pagnoux, K. Westman
   a) European Vasculitis Genetics Consortium ..... D. Jayne
   b) VCRC/North America genetic studies ..... P. Merkel
   c) Long-term outcome of EUVAS studies ..... L. Moi

2) CLASSIFICATION AND ASSESSMENT PROJECTS ..... A. Mahr, G. Tómasson
   a) ACR-EULAR Diagnosis and Classification Study (DCVAS) ..... P. Grayson, J. Robson
   b) ACR-EULAR AAV response criteria ..... G. Tómasson
   c) OMERACT ..... Direskeneli/Hatemi/Merkel/Robsor
   d) Revisions of classification of cutaneous vasculitis ..... C. Sunderkoetter
   e) Polyarteritis nodosa ..... O. Karadag
   f) Relapsing Polychondritis ..... P. Grayson

3) REGISTRIES ..... T. Ito-Ihara, M Little
   a) VCRC/Vasculitis Patient-Powered Research Network ..... P. Merkel
   b) European Reference networks ..... M. Little
   c) Studies of Interstitial Lung Disease in ANCA-associated vasculitis
      i) Japanese Study ..... K. Suzuki
      ii) North American/EU/Other study ..... L.F. Flores-Suarez, U. Specks

4) BIOMARKER & HISTOLOGY STUDIES ..... I. Bajema, P. Monach
   a) PEXIVAS ..... M. Walsh, M. Wester
   b) Histology studies ..... I. Bajema
   c) VCRC Biomarker and Tissue Repositories ..... P. Monach
   d) VCRC: Study of gene expression in cutaneous vasculitis (CUTIS) .... P. Grayson

【EUVAS-VCRCIM: April 7, 2019 Philadelphia, USA】

As shown in the agenda, European Vasculitis society/vasculitis clinical research consortium investigators meeting was held 9:00 a.m. Sunday April 7, 2019 in Loews Hotel, Philadelphia, USA. It was chiared by Prof. Peter A. Merkel (Pennsylvania University) and Prof. David Jayne (Cambridge University, UK). From Japan, 2 proposals: one was Japanese ARAMIS of Prof. Tamihoro Kawakami, the Tohoku Medical Pharmaceutical University (Dr. Toshiko Ito-Ihara: Kyoto Pref. Medical University) reported representation), and the Japanese Pulmonary limited-vasculitis by Prof. Sakae Homma, Toho University Medical School (Prof. Kazuo Suzuki, Teikyo University did representation).

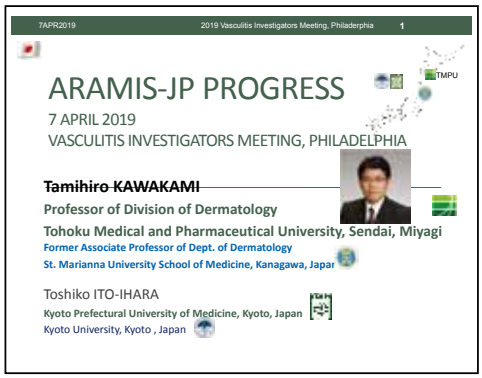
会場から見た街の風景

Prof. David Jayne（左） Prof. Peter Merkel（右）

左からProf. L.F. Flores-Suarez, Prof. U. Specks, Prof. K. Suzuki and Dr. T. Ihara

次回2021年第20回ANCA Workshopの会長を務めるProf. Mark Little（右）猪原先生（左）

日本からの２件の提案　　2 Proposals from Japanese Project Group



Stem Cell Therapy Consortium

帝京大学アジア国際感染症制御研究所：ステムセル治療コンソーシアムブランチ規程（2019年7月1日付）

構 成 員

ブランチ長：鈴木和男

帝京大学教員　三牧正和：医学部小児科　教授
白藤尚毅：医学部内科（血液内科）教授 ＋ 治療スタッフ
吉岡　昇：医学部生理学講座　講師
寺本民生：臨床研究センター　センター長（アドバイザー）

帝京大 ADC 研　協力者
岩間厚志：東京大学医科研　幹細胞治療研究センター幹細胞分子医学　教授
長村登紀子：東京大学医科研附属病院　セルプロセッシング・輸血部　部長
原田浩徳：東京薬科大学生命研　教授
小澤敬也：自治医科大学免疫遺伝子細胞治療学、東大医科研附属病院　前院長
岡崎富男：重症心身障害児施設　ときわ呉　施設管理責任者
布井博幸：重症心身障害児施設　愛泉会日南病院　院長
荒戸照世：北海道大学病院臨床研究開発センター　教授

国外
ベトナム　Vinmec Research Institute of Stem Cell and Gene Technology (Vinmec International Hospital)
Nguyen Thanh Liem, Director
米国　NIAID-NIH Deputy Chief, Lab. of Clinical Immunology and Microbiology
Harry Malech: Chief, Genetic Immunotherapy Section
NICHD-NIH
Keiko Ozato: Chief, Section on Molecular Genetics of Immunity

基礎実験の国際共同研究の推進

脳性麻痺（cerebral palsy, CP）の回復機構を解析する。自家骨髄単核球髄腔内移植（BMMNC-IT）により、性や年齢、粗大運動能力のレベルに関係なく、等しく効果的であることがベトナム Vinmec RISCGT の Liem 所長らにより報告されている（Nguyen et al, 2017）。これらの事例は、単独施設の報告ではあるが、70例にのぼる多数の臨床試験が行われ、安全性にも配慮されている。その事例を下図に示す。

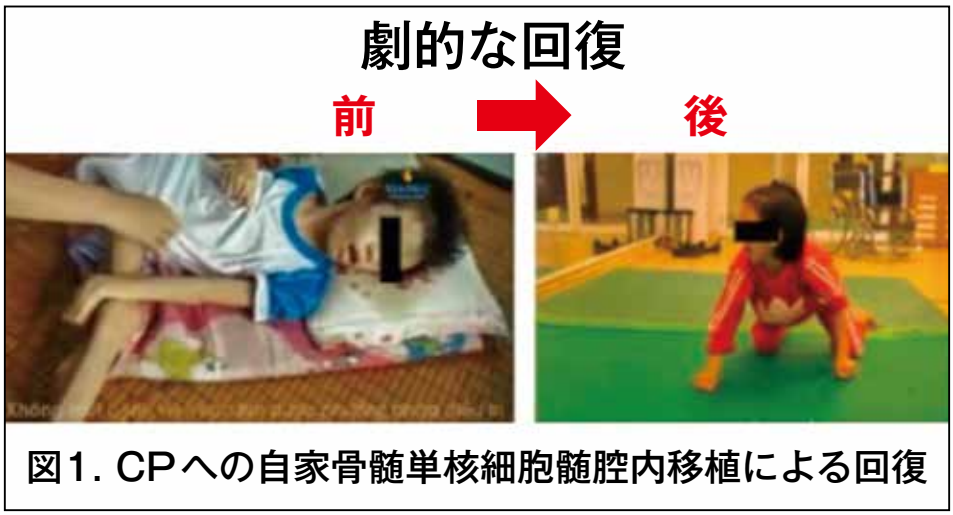


図1. CPへの自家骨髄単核細胞髄腔内移植による回復

CP 回復機構の解析については、小児脳機能の分子機構について長年の研究リーダーである米国 NIH/NCCHD の Dr. Ozato と共同研究を行うとともに、Vinmec RISCGT の Liem 所長とは行動研究、臨床データ解析を共同で行う。

医療技術等国際展開推進事業に初めて参加した経験と社会貢献 in ベトナム

帝京大学医学部附属病院ME部　係長　臨床工学技士・臨床検査技師　川﨑　義隆

Experience for the First Time in Medical Development and Other International Development Promotion Projects and Social Contribution in Vietnam

Yoshitaka Kawasaki, Master's Degree
Teikyo University School of Medicine, General Manager,
ME Department Clinical Engineer and Laboratory Engineer

The project for promoting international expansion of medical technology, etc. involves dispatching international issues, persons who have insights on Japan's medical policy and social security system, etc., Japanese medical workers and engineers of medical related industries to related countries. We will accept medical professionals and health and medical personnel from foreign countries. Through these activities, we will promote the growth of Japan's medical field by sharing experiences related to Japan's medical system such as the public medical insurance system of Japan, transferring medical technology and promoting the international development of high-quality Japanese pharmaceuticals and medical devices. At the same time, we aim to bring about a virtuous circle for both Japan and developing countries by enhancing Japan's trust in the international community by contributing to the improvement of public health and medical standards in the partner country.

偶然からの始まり

今回この事業に参加することになった経緯は、たまたま３年前のある研修会で７年前まで当院で働いていた同僚（現在、国立国際医療研究センター病院勤務）に久しぶりに会い談笑している中で、今回の事業に参加を考えている話を聞き、私自身が国際（社会）貢献に以前から興味があったのもあり、是非とも帝京大学病院とコラボレーションしてアジアに進出しましょう、と軽い誘いで承諾してしまったのがきっかけであった。詳細を聞いてみると、日本政府の「日本再興戦略」として、新興国や途上国等に対してアウトバウンドの基盤となる保健サービス・システムの強化支援を行うことを目的に、医療国際等展開推進事業があり、この医療国際展開の施策として、各国保健省との協力関係構築を通じて、我が国の先端医療についての技術移転や、公的医療保険制度に関する知見や経験の移転などを推進していくこと、2017年度事業のうちの一つとして“アジアにおける放射線・臨床検査・ME部門の技術支援事業”があり、この中でMEチームは“ベトナム国における医療機器管理の現状と対策調査および技術移転事業”をベトナム、ハノイの国立バックマイ病院というベトナム国内最大施設で展開するということであった。とは言っても、私は支援事業も初めてであり、まして行ったこともない海外のベトナムである。どんな国なのかネットや訪問したことのある人達に話は聞いてみたがあまりピンとこず、行けば何とかなると、現地で行う予定のセミナーの準備などを行いいざ出発となった。

いざベトナムへ

このような経緯でベトナム（越国）に行く機会を得た。東南アジアには人工透析材料の工場見学でタイ、旅行でシンガポールには行ったが、ベトナムは初めてであった。

少なからず不安も抱きながら、一昨年６月ハノイに降り立った。そして、不安は的中した。ハノイ市内には信号は数えるほどで、よく言うと譲り合いの精神で成り立っているが、人はクルマが途切れそうなタイミングを見て、道を一定速度で渡り始め、逆

側の交通状況を観察しつつ渡り切る。バイクやクルマは、それでもすり抜けようとするので、歩くスピードを急に変えたり、中途半端なところで急に立ち止まったりすると、逆にクルマやバイクが当たってくる可能性があり要注意だ。また、クルマやバイクは容赦なくクラクションを鳴らす。ただし、“どけ～！”という意味でなく“すぐ近くにいるから気をつけて！”という意味らしい。道路事情はさておき、その道わきで野菜に南国フルーツまでは良いが、生肉に魚と露天商が賑わう。ふと見上げると、電柱や軒下には無数の電線が絡み合って、どれがどれやら状態であり、カルチャーショックの連続であった。

現地視察と日本での研修

さて、こんな少し引けてしまうような驚きの中、いよいよベトナム最大のバックマイ病院到着である。院長らに迎えられ、あいさつの後に本題の日本の医療機器管理室にあたる“医療資材部”へ副部長のVan氏とともに移動し、彼らの業務や院内の機器管理状況や説明を受ける。簡単なレントゲン撮影機から血液検査の遠心分離機まで、ありとあらゆる機械の修理を頼まれ、ガス配管やボイラー、院内のエアコンや電気配線まで担当するとのこと。当院にたとえるならME部・中央放射線部・中央検査部・管財課・防災センターの業務を行っているようなものである。しかし、機器の台帳化はなされず、どれがいつ購入されたか？何回目の修理なのか？前にどこを修理したかの記録もない。部屋の前の広い廊下には、いつから壊れて、どのくらい放置されているのか想像もつかないような機器が厚いほこりの下に山積みされている。ただし、病院中枢に捨てるための報告をしなければならないため、これらに関してはしっかりとExcelで一覧表になっていた。続いて、院内見学である。広い敷地の中は人であふれかえり、日陰では日本流に言うとゴザを引いて昼寝したりお弁当食べたり、タバコ吹かしてたむろしている。院内の廊下も人ひとヒトである。蒸し暑い外より人気があるようで、弁当組がストレッチャーが通るたびに立ち退きを食らうも、すぐにまた宴会状態に復する。どうも患者の家族らしい。そんな雑踏をかき分けて汗だくになってICUに到着。そこで目にしたものは、雑然とした人工呼吸器の森である。私が30年くらい前の新人時代に見たような“Puritan Bennett”や箱型レスピ、まさに博物館である。Van氏は、今も動くと自慢げには解説するものの、もちろん誰にいつ使われたのかは把握できていない。こんな彼らも何とかしたいという思いがあった。ただお手本もないので方策がわからない。何とかしてあげたいという思いで4か月後にVan氏の研修を国立国際医療研究センター病院（以下NCGM）を中心に帝京大学病院でも受け入れ、日本での一般的大病院・大学病院の医療機器管理体制を学んでいってもらった。日本で目にしたものは、きっと彼にとっても驚きであったろうが、出来そうなことを探し何かから始めることを約束し、5日間の研修を終えて帰国した。

そして2か月後に今回の事業評価目的に我々が再訪することになった。Van氏らの努力の甲斐あって、なんとガラクタの山はきれいに片付いて、人工呼吸器にもシリンジポンプにも背番号が貼られ、整然と並んでいた。すごい変わり様であった。ちょうど時期を同じくして、病棟も隣の新病棟に移ったことも良いタイミングだったとは思うが“医療資材部”の努力には脱帽した。加えて、周辺地域の病院スタッフや教育機関の教官や学生、計150人が集い“日越医療機器管理セミナー：安心、安全な医療提供に向けて”も盛況のうちに開催できた。その夜、酒を飲みながらVan氏に“やれば、できるじゃん”と肩をたたくと、満面の笑みを浮かべていたのが忘れられない。ちょっとしたアドバイスと学ぶ機会があれば、自分たちだけでも何とかしようというエネルギーと実行力を伴うパワーを感じたハノイの夜であった。

これまでは日本も含めて先進国からの多大な援助が物や建物として入った。現にバックマイ病院も日本の援助で建ち、日本でもおなじみの透析器や人工呼吸器などがJICA経由などで供与され、我々日本人に感謝していたが、もらうことに慣れてしまい自分たちで何とかしようという気持ちはどこまであるのかと不安もあったが、今回経験

したように、彼らも変わろうとしたい思いが強く、そんなソフト面からの援助の重要性を強く感じた。

30数年前、目覚ましい医療進歩に伴い高度な“医療機器”が多く出現し、とても“医療用具”のレベルで済まされなくなり、日本では1987年に臨床工学技士法が制定され、国家資格として医療機器管理という概念が広まった。それまでは、透析や人工心肺などは看護師、臨床検査技師、理学部卒の実験助手などが担当していたのを“臨床工学技士”としてまとめた。

ベトナムでも“Decree 36 on medical equipment management”という法令が2016年に制定された。日本の医薬品医療機器等法（薬機法）に相当するが、残念なことに実効には至っていない。しかし、これからベトナムでも関係者が学び、考え、そして自国に合った制度やスタイルに築いていくことであろうと確信を得た。

今回は偶然からはじまり驚きがあり感動と希望を感じさせてくれた事業であった。試行錯誤を繰り返しながら何とか結果が残せ、Van氏をはじめベトナムの方々には本当によくしてもらい、喜んでもらい少し肩の荷が下りた感じである。今後も大学病院ME部として医療技術等国際展開推進事業に参加し、可能な限り国際支援事業を通して貢献したいと考えている。

思わぬサプライズ

NCGMが長年にわたり地道に行ってきた国際医療支援・協力の取り組みが評価され、WHOの保健医療賞を受賞した。

この賞、「アラブ首長国連邦保健基金賞」（UAE Health Foundation Prize）は、保健開発に対してとび抜けて優れた貢献をした個人、機関、組織に与えられるものである。

今般、同賞の選出委員会は、全会一致で2019年の「アラブ首長国連邦保健基金賞」について、国立国際医療研究センター（NCGM）とアスクワル・ヒロンガ医師（タンザニア）に授与することになった。アラブ首長国連邦保健基金賞表彰は、5月の第72回WHO総会で行われ、授賞式は、5月24日（金）、スイス・ジュネーブのWHO本部で行われた。NCGMからは、國土典宏理事長が代表として授与された。

同賞はかつてヒラリー・クリントン氏やビル＆メリンダ・ゲイツ財団等も受賞している。

このようなすばらしい賞をいただける事業に参加出来たことを大変誇りに思うとともにご協力下さった方々に心より感謝申し上げたい。

謝辞

最後に、このような貴重な機会を下さいました、国立国際医療研究センター病院臨床工学科部長 保坂　茂先生、国際医療協力局の土井正彦さん、臨床工学科 小川竜徳さん、快くベトナム出張をご承諾して頂いた帝京大学医学部附属麻酔科主任教授・ME部長 澤村成史先生、出張中業務をフォローしてくれたME部の同僚、帝京大学アジア国際感染症制御研究所（ADC研）鈴木和男先生ほか関係各位に心より感謝いたします。

TAVP-Training for Students

August 18-25, 2019

医学部５年生：衛生学公衆衛生学実習「1.ベトナム感染症」
東南アジア・ベトナムでの感染症の実態調査・見学（TASP-TAVPとの連携プログラム）

実習概要

臨床実習、国際保健・予防医学、医療システムやアクセスの観点も含めて学習

渡航期間　2019年8月18日～25日

研修先

・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
・ハノイ郊外の病院：感染症実習
・Vinmec国際病院、日本大使館、JICA、他

付添教員

鈴木和男、河内正治、高橋和浩、玉井大地、鈴木章一

左から Takayoshi Okubo, Yuka Nagamatsu, Usam Kim, Rei Hirano, Rinko Iwase, Moe Kajita, Shinji Takada, Kazuo Suzuki, Hideya Tanaka

TASP PLAN SAKURA Science Plan 2019

日本・アジア青少年サイエンス交流事業
さくらサイエンスプラン

帝京大学アジア国際感染症制御研究所（ADC研）は、今年度もさくらサイエンスプランに採択されました。2019年10月28日から11月６日の10日間、ベトナムから８名の研修参加者を招いて、科学技術研修コース「安全管理」「感染制御」「危機管理」「シミュレーション」の４つをテーマに実施致します。さくらサイエンスプランとは、産学官の連携により、アジアなどの若者を日本に招へいし、日本の科学技術を体験する事業で、2014年からJSTが行っています。ADC研では、2015年から採択されており、今年で４回目の実施となります。

なお、鈴木和男所長は、2019年５月にベトナムを訪問した際、今回来日予定の実習生の病院を訪問してきました。

ADC was adopted by the Sakura Science Plan again this year, inviting eight trainees from Vietnam from October 28 to November 6, 2019 (10 days). The four themes of the science and technology training course includes “Safety Management”, “Infection Control”, “Crisis Control” and “Simulation” will be implemented. The Sakura Science Plan is a project where young people from Asia are invited to Japan in cooperation with industry, academia and government, and experience Japanese science and technology. JST has been implemented the project since 2014. ADC Labs has been adopted by the project since 2015, and it will be the fourth time this year.

When Dr. Kazuo Suzuki visited Vietnam in May 2019, he visited trainees who will be visiting Japan.

研修予定者8名（ハノイ、ホーチミン）：2019/5/20-22 現地ヒアリング

Hanoi Vietnam National Children’s Hospital

Mr. Hieu Trung Do, Infectious Disease Dept.
Dr. Van Tran Th, Cardiology Intensive Unit.
Dr. Dao Nam Huu, Infectious Disease Dept.

Hanoi Vietnam National University

Mr. Bui Son Nhat, Dept. of Pharmacology

Hanoi Medical University

Dr.Doan Thu Ha,
Emergency Dept.

Ho Chi Minh Children’s Hospital 1

Dr. Chau To Uyen, Gastroenterology Dept.
Dr. Pham Quynh Mai Trang, Neonatal ICU.
Dr. Tran Van Cuong , Emergency Dept.

2017, 2018のSAKURA Science
参加者たちも交えて懇談

Dr. Tuanにデング熱の説明文書の
掲示について説明を受ける

医学部6年生の海外Bedside Clerkship（BSC）実習

帝京大学医学部では、6年次にクラークシップ制による選択制臨床実習［Bedside Clerkship（BSC）］を行っています。今年度も5年次にベトナム感染症実習に参加した学生の中から、BSCを海外で実践したいとの希望が出ました。海外実習に強い希望があり、海外BSCが可能な2名を選び、学長、副学長の許可を得て実施しました。今回実習に参加した、笠井健司さんと長久大介さんに経験談や感想を寄稿してもらいました。

Bedside Clerkship (BSC) by 6th grade medical school students

In 2018, 5th grade students have studied “the infectious disease practice in Vietnam” and learned a lot about the real status of the sick patients in Vietnam. Actually some of the students really wanted to try on BSC overseas in their 6th grade. So we selected 2 applicants (Mr. Kenji Kasai & Mr. Daisuke Chokyu) and sent them to the USA and UK. Now they share us their experiences and impressions.

報告 1

Joslinでの経験を振り返って

帝京大学医学部医学科6年　笠井健司

実習期間　2019年5月13日～2019年6月7日

私は2019年5月13日から6月7日までの間、米国ボストンにあるJoslin Diabetes Center（以下Joslin）にて臨床実習を行ったので報告する。Joslin はHarvard Medical School（以下Harvard）の附属機関で糖尿病の研究、臨床の双方における世界的権威である。また医学教育に注力している医療機関としても非常に有名であり、世界各地から医学生、医師やコメディカルを受け入れている。私の受け入れにご尽力頂いたC. Ronald Kahn教授に実際にお会いでき、外来見学をはじめ最先端の研究内容を教わったり患者に対する治療プログラムを体験したりすることができた。

外来の見学は非常に実りの多いものであった。渡米前にHarvardの医学生プログラムの責任者の1人である、Nuha EL Sayed先生に私の実習予定表を作成して頂いた。糖尿病の通常の外来だけでなく腎臓や眼科の特殊外来も見学させて欲しい旨伝えたところ快く承諾して下さり、糖尿病患者を多角的に診る視座が得られた。外来見学で師事した先生方の中には難民のための糖尿病の疫学的対策を行っている方や最新の研究を平行して行っている方がおり、外来の合間に説明を受けることができた。また医師の外来だけでなくコメディカルの外来も見学した。JoslinにはNurse Practitionerが多数在籍しており、外来で医師と同様に患者を診察し処方箋を発行していたのが印象的であった。外来の午前と午後の部の合間にはGrand Round Lectureが開催され、研究者の成果発表や他大学の教授の講演を聴講した。

Joslinでは患者教育も積極的に行っている。4日間連続して行うDiabetes Outpatient Intensive Treatment Program（DO IT）に全日参加し、日々の生活の見直しや運動療法、栄養学の知識を患者と一緒に学んだ。我が国ではこの様な手厚い教育は行われていないと考えられるので、世界的な流れとして病気を患う前や患った後の悪化の予防に注力していることをより多くの人に伝えたい。また、実際に持続血糖測定器を体に取り付けて患者と同様に10日間測定を行い、毎食における炭水化物の定量の煩わしさや着用時の違和感を体験した。

今回の実習を通して、内分泌領域について新たな知見が得られただけではなく、米国の医療事情についても学ぶこともできた。これらについて我が国の医療関係者等に伝えていくことはJoslinで研鑽を積ませてもらった者の責務だと考えている。

謝辞

今回の実習は、帝京大学学長の冲永佳史先生、帝京大学アジア国際感染症制御研究所所長の鈴木和男先生、帝京大学臨床研究センター長の寺本民生先生、Joslin Diabetes Centerの先生方の支えなくしては成り得なかったことであり、深く感謝いたします。

Looking Back My Experience at Joslin

Teikyo University School of Medicine, Tokyo Kenji Kasai


Joslin Diabetes Center

I'm reporting my clinical clerkship at Joslin Diabetes Center (Joslin), Boston, USA from 5/13/2019 to 6/17/2019. Joslin, a part of Harvard Medical School (Harvard), has been world leading institution of research and clinical practice. It has also been very famous as a medical institution that makes an effort on medical education. It has been accepted medical students, doctors and co-medicals from all over the world. I could have an honor to see Prof. C. Ronald Kahn who kindly supported me to come there. I could enjoy following Joslin's doctors at consultation rooms, learning latest studies and experiencing programs for patient's treatment.

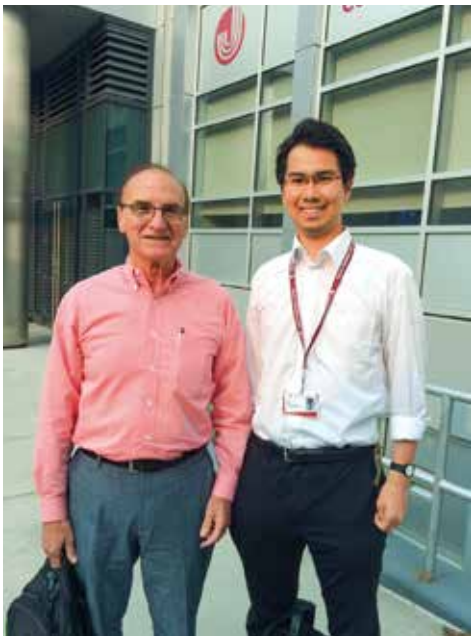
My experiences at consultation rooms were remarkable. Before I came to USA, Dr. Nuha EL Sayed, one of the program director of Harvard, kindly made my schedule at Joslin. When I asked her for an opportunity to follow nephrologists and ophthalmologists, she was willing to adjust my schedule so that I could see patients in various ways. There were a lot of doctors who had unique backgrounds, for example, a doctor who had made an effort to help refugees with diabetes with epidemical measurements and a doctor who had done latest researches above all others, and they told me their stories. I also had a chance to follow to co-medicals. There were a lot of Nurse Practitioners at Joslin, and I was impressed that they treated their patients and prescribed like doctors. At lunch time, between morning and afternoon parts of outpatient, Grand Round Lectures were held for medical scientists to give presentations of their studies, and sometimes I had opportunities to listen to professor's lectures from other universities.

Joslin has also made an effort on patient education. I joined "Diabetes Outpatient Intensive Treatment Program (DO IT)" through 4 consecutive days, and learned how to review patient's life style, exercise and also learned nutritional therapies. I would like more people to know that it is world trend to prevent illness because I assumed patient education was not very common in Japan. I also tested Consecutive Glucose Monitoring for 10 days, and learned how difficult carbon count and keeping it on body were.

In this visiting, I could learn not only latest studies on endocrinology but also medical circumstance in USA. It may be my duty as a trainee at Joslin to tell these kind of things to other medical staff in Japan.

Acknowledgement

This visit to Joslin was supported by Pres. Yoshihito Okinaga (President of Teikyo University), Prof. Kazuo Suzuki (Asia International Institute of Infectious Disease Control, Teikyo University), Prof. Tamio Teramoto (Teikyo Academic Research Center), and staff at Joslin Diabetes Center.


With Prof. C. Ronald Kahn

At Harvard Medical School

報告 2

Cambridge大学留学を終えて

帝京大学医学部医学科6年　長久大介
実習期間　2019年5月13日～2019年6月7日


With Prof. David Jayne

私は2019年５月13日から６月７日の４週間、Addenbrooke's Hospitalの腎臓内科Lupus and Vasculitis Departmentで実習させていただきました。

私が留学を希望した理由として昨年度のベトナムでの公衆衛生実習が挙げられます。ベトナムでは日本との様々な医療や文化の違いを学ぶことができ、私の人生の中でも貴重な経験となりました。またこの素晴らしい実習を機に幅広い視野を持つ国際的な医師を志すようになりました。しかし、この留学時はADC研究所の鈴木教授をはじめ多くの先生方や仲間たちのサポートがあり私一人では何もできませんでした。そのため今回は自分の中で何か大きなことを成し遂げたいと強く思い、鈴木教授にイギリスでのプログラムを提供していただきました。またAddenbrooke's Hospitalでの所属先が腎臓内科の特に免疫疾患を主に取り扱っているチームであったこと、イギリスではGP制度が確立されえており大きな病院では難しい様々な疾患を経験できることなどが留学を決めた理由です。

私が実習させていただいたAddenbrooke's Hospitalは250年前に開院したCambridgeの南に位置する歴史ある大学病院です。そのため医師をはじめ医療従事者の数も多く、腎臓内科も透析チームや移植チームが独立しています。その中でも私は鈴木教授の昔ながらのご友人であるDavid Jayne教授が率いるLupus and Vasculitisチームに所属させていただきました。Jayne教授はANCA関連血管炎のスペシャリストであり、様々な患者さんがJayne教授の診察を受けに世界中から集まります。そのためAddenbrooke's Hospitalは日本ではあまり出会うことができない疾患も経験することができました。

Addenbrooke's Hospitalでの実習内容としては外来見学はじめカンファレンスや研究ミーティング、勉強会、病棟見学など多岐に渡る経験をさせていただきました。イギリスでは日本と異なり患者さんはまずGPという、かかりつけ医の診察を受けてから紹介状を持って大学病院へ来院するという医療制度が確立されています。そのため外来見学では既に診断がついたSLEやベーチェット病、ANCA関連疾患、巨細胞性動脈炎、IgG４関連疾患など複雑な免疫学的疾患症例を数多く経験することができました。Vasculitisクリニックでは３人に１人はANCA関連疾患の１つであるGPA（Granulomatosis with polyangiitis）の患者さんです。日本ではMPA（Microscopic polyangiitis）の患者さんが多く、一方でGPAの患者さんは3,000人以下で普段あまり見かけません。これらの疾患は日本とイギリスでは真逆であり、医師になる前にこのような症例を経験することができ大変勉強になりました。ANCA関連疾患の治療は主にステロイドとトシリズマブという日本で開発された免疫抑制薬で、比較的コントロールできている患者さんが多かったですが、呼吸器症状や皮膚症状が出てしまい腎臓の機能が低下し透析や移植を導入している患者さんも一定数おられました。しかしこのような患者さんでも、決して諦めないことが大切なのよ、と先生が語ってくれたことがとても印象的でした。

また、Addenbrooke's Hospitalの関連施設であるCambridge Dialysis Centreでも実習させていただきました。透析患者さんのコントロールに関しては日本との違いはあまりありませんが、とても大きくきれいな施設で学ぶことができました。私が見学させていただいた透析外来では先生が「日本人はシャント手術がとても上手です」と患者さんに説明していて、私は日本人の医学生として誇りに思いました。

イギリスと日本では医療制度が大きく異なっており医療費はほとんど無料です。また免疫疾患は多くの薬を処方するため、外来では細かい問診や身体診察で無駄な処方をしないようにすることが患者さんのためでもあり医療費を抑えることにもつながると先生が言っていました。また外来終了後に先生方が集まりその日のフィードバックを行いみんなで情報を共有していたことも日本ではあまり行われていないと思います。そして私が一番感じたことは、もちろん文化の違いもありますがイギリスの医療現場は日本より医師と患者の距離が近いように感じました。

私はこの実習をさせていただくためにホームステイを選択しました。もちろんイギリスでの生活は日本とは違います。ホームステイを通してイギリスの文化に触れ、また同時に日本人としての自分を改めて見つめなおす良い機会となりました。しかし一方で１か月間毎日楽しかったわけではありません。伝えたいことが伝わらずまた英語が聞き取れないこともあり悔しい思いをする日も多々ありました。その中で先生方やスタッフの皆さん、ホストファミリーにとても親切にしていただき、皆が経験することができない素晴らしい旅を乗り越え、終えることができま

した。医療の現場でもグローバル化が当たり前になってきており、これからは留学の壁も段々と低くなっていくのではないでしょうか。もし少しでも留学に興味を持っているなら挑戦してみるべきです。医学生の間に留学することは、自信がつき医師として必要な感性を獲得することができると思います。私はこの貴重な体験を日本の医療現場に還元して、また遠くない将来、今度は医師として再び留学に挑戦したいと思います。最後になりましたが、この留学をサポートしていただいた鈴木教授並びにAddenbrooke's Hospitalの先生方や医療スタッフの皆さまに心より御礼申し上げます。


Addenbrooke's Hospital

Clinical Training at the University of Cambridge

Teikyo University School of Medicine, Tokyo　Daisuke Chokyu

As a medical student in a 6th grade at the Teikyo University, I practiced at the Lupus and Vasculitis Department at Addenbrooke's Hospital for four weeks from May 13 to June 7, 2019.

One of the reasons why I wanted to study abroad was the practice of public health in Vietnam last year. In Vietnam, I could learn about various medical and cultural differences from Japan, and it was a valuable experience in my life. So I wanted to become an international doctor with a broad perspective, taking advantage of this wonderful practice. However, at the time of the study abroad, I could not do anything with the support of doctors and friends, including Prof. Suzuki of the ADC Research Institute. For this reason, I really would like to accomplish something big for myself in this time, and Prof. Suzuki offered a program in England. Another reason was that Addenbrooke's Hospital's affiliation was a team mainly dealing with immune diseases, especially in kidney medicine, and the GP system has been established in the UK, and it is possible to experience various diseases in a large hospital.

Addenbrooke's Hospital where is a historic university hospital located south of Cambridge, which established 250 years ago. So, there are a large number of doctors and healthcare workers, and the dialysis and transplantation teams are independent of the renal medicine department. I had been belonging to the Lupus and Vasculitis team led by Prof. David Jayne who is Prof. Suzuki's old friend. Prof. Jayne is an ANCA-related vasculitis specialist, and various patients come from all over the world to see Prof. Jayne. As a result, I was able to experience diseases that could not be encountered in Japan.

The training contents at Addenbrooke's Hospital included a variety of experiences, for example outpatient care, conferences, and research meetings. Unlike in Japan, in the UK a medical system has been established in which patients first receive a medical examination from their general practitioner, and then visit the university hospital with a referral letter. As a result, I was able to experience many cases of complex immunological diseases such as SLE, Behcet's disease, ANCA related diseases, giant cell arteritis and IgG4 related diseases. At Vasculitis Clinic, there are many patients with GPA (Granulomatosis with polyangiitis), one of the ANCA related diseases. In Japan, there are many patients with MPA (Microscopic polyangiitis), while less than 3,000 patients with GPA. These diseases were the opposite in Japan and the United Kingdom, and I was able to experience such cases before I became a doctor, and I studied very much. The treatment for ANCA-related diseases is mainly steroids and tocilizumab, an immunosuppressant drug developed in Japan, and there are many patients who can be relatively controlled, but if respiratory symptoms and skin symptoms appear and kidney function declines, patients must dialyze and transplant. And my teaching doctor told me that it is important that even in case of such patients we could never give up.

We also practiced at Cambridge Dialysis Center, a related facility at Addenbrooke's Hospital. There was not much difference with Japan about the control of dialysis patients, but I was able to learn in a very large and beautiful facility. When I visited at the dialysis outpatient, my teaching doctor explained to the patients that "Japanese doctors are very good at shunting operation." and I was proud as a Japanese medical student.

The medical system was very different in Britain and Japan, and the medical expenses were almost free of charge. In addition, doctors gathered after the end of the outpatients care to give feedback and share information with everyone. I think that it is not often done in Japan. Also in the UK I felt that the distance between doctors and patients was closer than that in Japan.

I chose a homestay for this training. Of course, life in England is different from Japan. It was a good opportunity to get in touch with the British culture through homestay, and at the same time re-evaluate myself as a Japanese. But on the other hand, I did not enjoy it every day for a month. Sometimes I could not speak and hear English. The doctors, staff, and host family were very kind, so I was able to finish a wonderful trip that everyone could not experience. Globalization is becoming commonplace even in the medical field, and the barrier to studying abroad will gradually decline from now on. If you are interested in studying abroad, you should try it. I think that studying abroad as medical students can give you the confidence you need as a doctor. I would like to return this valuable experience to the medical field in Japan and try to study abroad as a doctor in the near future. Last, I would like to thank Prof. Suzuki and Prof. Jayne and medical staff at Addenbrooke's Hospital for supporting this study abroad.

Detection of protein-bound 3-nitrotyrosine in the plasma of pediatric patients with severe ARDS and avian influenza virus infection

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Abstract

Nitric oxide (NO) and reactive oxygen species (ROS) may be involved in the pathogenesis of various diseases, including microbial infections, inflammatory diseases, and cancer. 3-Nitrotyrosine (3-NT) produced by NO and ROS is considered a biomarker of oxidative stress. Acute respiratory distress syndrome (ARDS) is an inflammatory lung disease and is associated with the excessive production of NO and ROS. Immunohistochemical analyses showed that 3-NT may be produced in the lungs of patients with ARDS. We have identified the extensive and NO-dependent formation of 3-NT in the lungs of mice with ARDS caused by the influenza virus (IFV). However, the biochemical and quantitative aspects of 3-NT formation in patients with ARDS remain poorly understood. Thus, we investigated the levels of plasma protein-bound 3-NT in pediatric patients with severe ARDS using a reverse-phase high performance liquid chromatography (HPLC) coupled with electrochemical detector (ECD). The plasma samples of 40 patients with influenza-negative ARDS (non-IFV-ARDS group) and of 7 patients with influenza-positive ARDS (IFV-ARDS group) were analyzed. IFV-ARDS group consisted of two patients with highly pathogenic avian influenza (A/H5N1) and 5 patients with seasonal influenza (A/H1N1 and A/H3N2). Twenty-five patients without ARDS were used as control (non-ARDS group). Patients in the IFV-ARDS group had significantly higher 3-NT levels (median: 0.350 μmol/mol) than those in the non-ARDS group (median: 0.210; $p = 0.046$). Moreover, the 3-NT levels were significantly higher in the non-IFV-ARDS group (median: 0.270; $p = 0.039$) than in the non-ARDS group. However, the difference was not significant, the survivors had higher 3-NT levels than non-survivors, and the 3-NT levels were higher in patients without multiple organ failure (MOF) than those with MOF. Moreover, the survival rate was more likely higher in the high 3-NT level group than in the low 3-NT level group, indicating the protective role of NO/ROS in the pathogenesis of ARDS. Using this method, we could successfully detect 3-NT from the plasma of patients with ARDS. This method is convenient, specific, and sensitive for 3-NT quantification that is applicable on clinical specimens; hence, it may help in the further understanding of the pathological roles of NO/ROS formation in ARDS.

Keywords: 3-nitrotyrosine, ARDS, highly pathogenic avian influenza, nitric oxide, reactive oxygen species

Introduction

Acute respiratory distress syndrome (ARDS) is one of the most serious inflammatory lung diseases in the intensive care field, and the rate of mortality from ARDS is still high to date[1,2]. Excessive production of inflammatory cytokines is triggered by several biological stresses, such as pneumonia, sepsis, and trauma, which damage alveolar epithelial and endothelial cells, and this phenomenon leads to respiratory failure via non-cardiogenic pulmonary edema[3,4]. ARDS triggered by an infection caused by a highly pathogenic avian influenza (H5N1) virus infection is particularly fatal, with an extremely high mortality rate of 60%[5-8]. Oxidative stress is greatly involved in the molecular pathology of fatal ARDS[9]. Nitric oxide (NO) and reactive oxygen species (ROS) may be involved in the pathogenesis of different diseases, including various microbial infections, inflammatory and neurodegenerative diseases, and cancer[10]. Particularly, in inflammatory conditions, NO is excessively produced by the inducible isoform of NO synthase (iNOS) from inflammatory cells, such as macrophages or neutrophils[11]. Accumulated evidence indicates that NO- and ROS-derived reactive nitrogen oxide species (RNS), such as peroxynitrite ($ONOO^-$) and nitrogen dioxide (NO_2), also have a pathogenic potential in various diseases[12]. Based on a previous analysis that used a murine model of ARDS caused by the influenza virus (IFV), 3-nitrotyrosine (3-NT) and 8-nitroguanine-related compounds, which are the nitration products of amino acids and nucleobases, are shown to be produced in the infected locus depending on excessive NO production associated with the induction of iNOS using immunohistochemical staining and high-performance liquid chromatography (HPLC)-electrochemical detection (ECD) method[13,14]. 3-NT is a chemically stable substance produced from the nitration of tyrosine residues of proteins, which are produced by RNS that are generated by the reaction of NO and ROS[15] or by NO_2 produced from nitrite (NO_2^-) by neutrophil myeloperoxidase[16]. 3-NT is used as a biomarker of oxidative stress[17]. To date, several studies have reported the use of immunohistochemical staining in locally producing 3-NT in the lungs of individuals with severe cases of pneumonia and ARDS[18,19]. However, analytical methods that use such antibodies are problematic in terms of specificity and have poor quantitativity, and such issues have been frequently observed[20]. Moreover, numerous studies have analyzed the lung tissues of deceased individuals during autopsy, and only few studies have analyzed the lung tissues of survivors because ARDS itself is a serious health condition and it is challenging to obtain lung tissues via biopsy due to the high invasiveness of the procedure, particularly in children and elderly individuals. On the other hand, blood is a biologic sample that can be obtained with relatively minimal invasiveness and can be collected at multiple points during the clinical course of the disease. However, reports about

successful detection of 3-NT using plasma are extremely limited. This may be attributed not only to the low level of 3-NT in the plasma but also to various contaminants in the plasma that lower the signal-to-noise (S/N) ratio, which causes a major barrier to the clinical application of plasma as a 3-NT biomarker. Therefore, in this study, we established a method for treating plasma proteins and optimized elution conditions of HPLC to develop an HPLC-ECD measurement system with high sensitivity and S/N ratio. Moreover, a quantitative analysis of 3-NT in the plasma proteins of patients with fulminant ARDS was performed, and the correlation between 3-NT levels and pathologic conditions was assessed.

Experimental procedures

Collection of plasma samples

The stored plasma samples of pediatric ARDS patients (aged ≥ 1 month) with or without IFV infection who were admitted at the National Hospital of Pediatrics, Hanoi, Vietnam (NHP), from December 2007 to December 2009 were retrospectively assessed in this study. Twenty-five plasma samples obtained from patients without ARDS were used as controls. The clinical and laboratory data of patients were also collected by reviewing the hospital records. The diagnosis of ARDS was made according to the Berlin definition, which includes acute onset within 1 week; bilateral opacities on chest imaging that is not fully explained by effusion, atelectasis, or the presence of nodules; respiratory failure not fully explained by cardiac failure or fluid overlord; and hypoxia (PaO_2/FiO_2 [P/F] ratio ≤ 300) treated with mechanical ventilation with PEEP ≥ 5 cmH_2O[21]. We enrolled patients with severe ARDS whose P/F ratio is ≤ 100 during the clinical course of the disease. A/H5N1 and seasonal IFV infection were confirmed with throat and/or nasal swab tested by reverse-transcriptase polymerase chain reaction at the hospital laboratory. The study was approved by the ethical committee of the National Center for Global Health and Medicine Japan on September 28, 2007 (approved number; NCGM-G-000449-00).

Sample preparation

Plasma (0.1 mL, which is equivalent to approximately 5 mg protein) was diluted with 0.1 M acetate buffer (pH: 7.2, 0.4 mL), and then plasma protein was precipitated by adding 0.8 mL of ice-cold ethanol and centrifuged at 3,000 x g for 10 min. The pellet was washed with ethanol/0.1 M acetate buffer (8:5 v/v, 1 mL) to remove nitrite and nitrate from the plasma and then dried. The dried pellet was resuspended in 0.1 M acetate buffer with 10 mM dithiothreitol and 1% SDS (pH: 7.2, 0.4 mL) and solubilized using a constant temperature incubator shaker (Microtube Maximizer Model MBR-024, Taitec, Japan) (160 rpm, 50ºC) for 24 h. Half of the solubilized protein (0.2 mL) was digested with 0.5 mg pronase (Calbiochem), which was dialyzed against nitrite- and nitrate-free 0.1 M acetate buffer (pH: 7.2), using the microtube maximizer shaker (160 rpm, 50ºC) for 24 h. The digested product was ultrafiltrated using Microcon YM-3 (molecular cut-off of 3,000 Da, Millipore, Billerica, MA) to remove undigested materials.

HPLC-ECD

A total of 20 µL of the ultrafiltrated sample was fractionated with an SC-50ODS column (3 mm x 150 mm, Eicom, Kyoto, Japan) using the HPLC-ECD system (PEC-510/HTEC-500, Eicom). The mobile phase was 200 mM phosphate buffer (pH: 3.0) containing 2% acetonitrile and 5 µg/mL EDTA, and the flow rate was 0.4 mL/min. The sensitivity and linearity of the HPLC-ECD system to 3-NT were verified by analyzing the serial dilutions of the 3-NT standard (Sigma), which ranged from 1 nM to 100 nM. The applied potentials for the dual-mode ECD were adjusted to achieve the highest S/N ratio between 3-NT and other components in the digested plasma proteins. The applied potentials were -800 mV for the reduction cell and +200 mV for the oxidation/detection cell. The detection limit of this system was approximately 1 nM (20 fmol) for 3-NT. The identification criteria for 3-NT were as follows: 1) identical elution time with authentic 3-NT, 2) increased peak by the addition of 3-NT standard to the sample, 3) and disappearance of the peak by reducing the reduction cell potential to -400 mV. The 3-NT levels were standardized by tyrosine levels, which were determined using an ultraviolet detector (SPD-10A, Shimadzu, Japan) connected just after ECD, and they were expressed as µmol 3-NT/mol tyrosine.

NO_2^-/NO_3^- levels in the plasma

To exclude the potential artificial formation of 3-NT by NO_2^-, plasma NO_2^- and NO_3^- concentrations were analyzed using the Griess reaction-based flow reactor system (ENO10, Eicom, Kyoto).

Statistics

PASW Statistics version 18 (SPSS, Chicago, IL, USA) was used for statistical analysis. A p value < 0.05 was considered significant.

Results

Characteristics of the patients

The clinical and laboratory data of the patients enrolled in the study were collected by reviewing hospital records and are summarized in Table 1. We analyzed the plasma samples of 40 patients with IFV-negative ARDS (non-IFV-ARDS group; group A) and of 7 patients with IFV-positive ARDS (IFV-ARDS group; group B). In group A, ARDS was caused by pneumonia in 22 patients and by sepsis in 6 patients,

Table 1. Summary of Clinical and Laboratory Data of the patients

Characteristics	Non-IFV-ARDS Group A	IFV-ARDS Group B	Non-ARDS (disease control)* Group C
Number	40	7	15
Age, year	0.32 (0.20-0.76)	7.00 (3.00-9.50)	1.33 (0.23-2.70)
Male, number (%)	16 (40.0)	5 (71.4)	11 (73.3)
Prognosis			
Death, number (%)	12 (30.0)	5 (71.4)	0 (0)
MOF, number (%)	21 (52.5)	2 (28.6)	0 (0)
Inflammation			
Body temperature onset, ºC	38.0 (36.8-38.5)	38.5 (37.5-39.0)	n/a
CRP, mg/dl	1.5 (0.6-4.9)	1.6 (0.5-3.2)	6.3 (6.1-15.4)
Respiratory parameters			
PaO_2, mmHg	59.5 (42.8-66.3)	58.6 (55.7-65.1)	62.6 (52.2-72.9)
$PaCO_2$, mmHg	43.5 (37.8-53.1)	41.0 (29.4-45.3)	34.5 (27.0-42.1)
Lowest P/F	44 (33-64)	38 (29-71)	n/a
Liver function			
AST, IU/L	83 (61-145)	209 (135-618)	123 (35-204)
ALT, IU/L	37 (26-72)	72 (40-194)	41 (37-141)
LDH, IU/L	932 (673-1249)	1276 (815-1914)	n/a
Blood cell count			
WBC, cells/µL	12000 (9500-19200)	4100 (3400-6700)	13800 (8660-18500)
RBC, x 10^3 cells/µL	3680 (3275-4315)	3980 (3925-4345)	4040 (3700-4810)
Plts, x 10^3 cells/µL	322 (213-499)	131 (117-211)	267 (29-382)
Days from onset to			
admission	5.0 (2.8-6.3)	5.0 (3.5-12.0)	n/a
sampling	7.0 (4.0-12.3)	4.0 (2.0-6.5)	n/a

Data are shown in median value (IQR) unless otherwise specified. n/a: not available.
*Group C included 15 patients with various diseases and 10 healthy individuals for a medical checkup.

whereas the etiology of ARDS was unknown in 12 patients. Group B consisted of two patients with highly pathogenic avian IFV (A/H5N1), 4 patients with seasonal IFV (A/H1N1), and 1 patient with seasonal IFV (A/H3N2). The median days (interquartile range, IQR) from onset of ARDS to sampling was 7.0 (4.0–12.3) for group A and 4.0 (2.0–6.5) for group B. In total, 25 patients without ARDS were considered as controls (non-ARDS group; group C). Group C included 15 patients with various diseases, such as anemia, idiopathic thrombocytopenic purpura, myocarditis, and fever of unknown origin, and the remaining 10 patients were healthy individuals who attended NHP for a regular medical checkup.

Detection of 3-NT in the plasma samples of H5N1-infected patients

A representative elution profile of plasma protein-bound 3-NT in patients with ARDS (H5N1-positive ARDS case) detected using HPLC-ECD are shown in Figure 1. Peak was observed at an elution time (24 min) identical with authentic 3-NT, and the peak disappeared by decreasing the reduction cell potential from -800 to -400 mV, indicating the specific detection of 3-NT. The lower panel shows the corresponding peak of tyrosine (9 min) determined using an ultraviolet detector.

The 3-NT levels of ARDS patients with or without IFV infection and controls

The 3-NT levels of each individual in all 3 groups are shown in Scattered and box plots (Figure 2). The mean levels of 3-NT in group A (0.54 ± 0.11 μmol/mol) and group B (0.98 ± 0.38 μmol/mol) were significantly higher than that of group C (0.21 ± 0.02 μmol/mol). Moreover, 5/40 (12.5%) patients in group A and 3/7 (42.9%) patients in group B showed high levels of 3-NT (≥ 1.0 μmol/mol). By contrast, none of the patients in group C showed 3-NT levels greater than 1.0 μmol/mol. Patients with ARDS had significantly higher 3-NT levels (median: 0.270 μmol/mol, IQR: 0.125-0.640, $p = 0.039$ in group A; median: 0.350 μmol/mol, IQR: 0.182-1.675, $p = 0.046$ in group B) than patients without ARDS (median: 0.210 μmol/mol, IQR: 0.122-0.278 in group C) (Mann-Whitney U test, vs group C). Although the median 3-NT level was slightly higher in group B than in group A, the difference was not significant ($p = 0.166$).

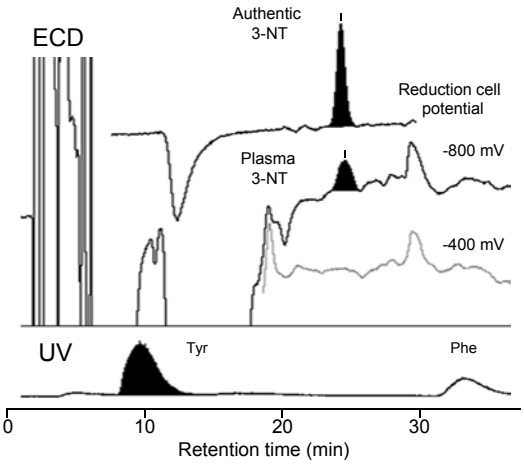

Figure 1. Typical elution profile of plasma protein-bound 3-NT in patients with ARDS detected using HPLC-ECD
Peak was observed at an identical elution time with authentic 3-NT, and disappeared by decreasing the reduction cell potential, indicating the specific detection of 3-NT. The lower panel shows the corresponding peak of tyrosine.

Logistic regression analysis for plasma 3-NT levels in patients with ARDS and controls

Compared to the control group (group C), the group with ARDS (group A+B) had significantly higher odds ratio (OR: 3.30, 95% confidence interval [CI]: 1.12-9.74, $p = 0.027$) for high levels of plasma protein 3-NT (≥ 0.3 μmol/mol, median of all 72 cases). Between the two groups with ARDS, group B had higher OR (OR: 4.42, 95% CI: 0.73-24.44, $p = 0.094$) for 3-NT than group A (OR: 3.17, 95% CI: 1.05-9.59, $p = 0.037$) (Figure 3). Next, groups A and B were compared via logistic regression analysis (Figure 4A). Results suggested that, compared with group A, group B had a higher 3-NT level with an OR of 2.26 ($p = 0.330$) where the level of 3-NT increases from the mean value (0.6 μmol/mol) of groups A and B. The risk of mortality in group B was 5.83 (0.99-34.38), which indicate a significantly worse prognosis than that in group A ($p = 0.035$). The ORs at which CRP and LDH had values greater than the median are more likely to be high (2.50 [0.43-14.51] and 2.71 [0.43-16.96], respectively). Although these results suggested that group B had higher inflammatory response and cytotoxicity than group A, the differences were

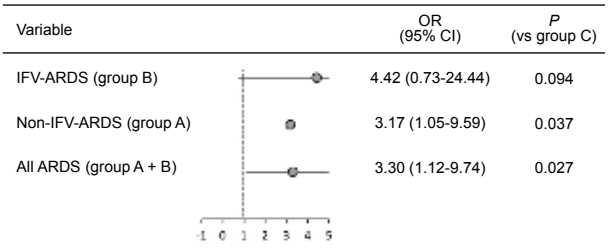

Figure 3. Logistic regression analysis for plasma 3-NT levels in patients with ARDS and controls
Compared to the control group (group C), the group with ARDS (group A+B) had significantly higher OR for high levels of plasma protein 3-NT (≥ 0.3 μmol/mol). Between the two groups with ARDS, group B had higher OR for 3-NT than group A.

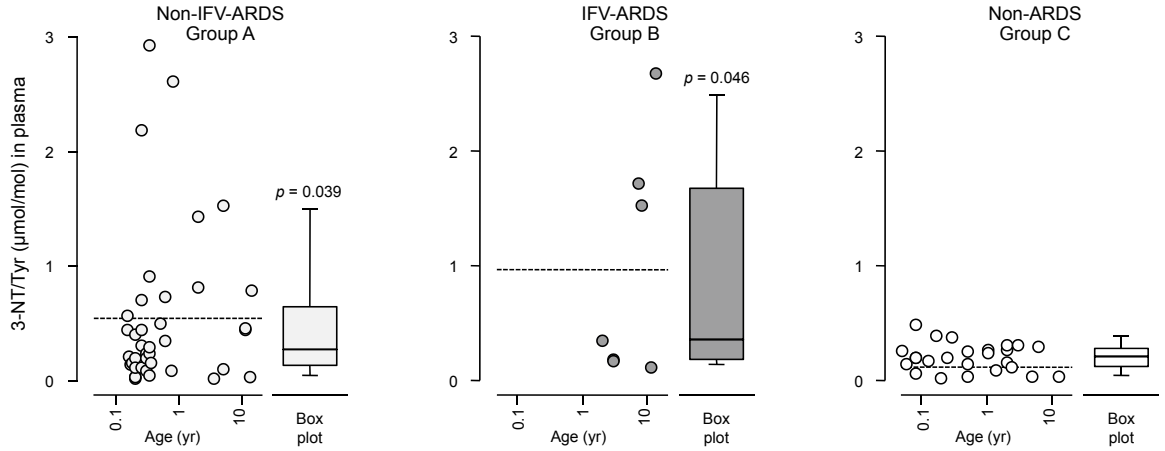

Figure 2. The 3-NT levels of ARDS patients with or without IFV infection and controls
The 3-NT levels in all groups are shown in Scattered and box plots. Patients with ARDS (Group A, B) had significantly higher 3-NT levels than patients without ARDS (Group C) (Mann-Whitney U test). Although the difference was not significant, the median 3-NT level was higher in group B than in group A. Dot lines show mean 3-NT level.

not significant (p = 0.296, 0.273, respectively). The risk of multiple organ failure (MOF) in group B was not significantly different from that of group A (p = 0.243), however, it was more likely lower than that of group A (OR: 0.36 [0.06-2.09]). This suggests that IFV infection is not systematic, rather localized in the lungs. The OR at which the white blood cell (WBC) count exceeded the median was 0.08 (0.01-0.77), thus showing a significantly decreasing trend (p = 0.010). This result suggests the presence of leukopenia, which is often observed in individuals with IFV infection. The examination of the survival curve revealed that the mean survival time (MST) was 35.9 ± 2.3 days (group A) and 14.3 ± 2.9 days (group B), and the survival rate of the group B was significantly lower than that of the group A (p < 0.001, Log-Rank test, Figure 4B).

NO_2^-/ NO_3^- levels in the plasma

The formation of 3-NT is possible via the nitration of tyrosine by NO_2^- in acidic conditions[22]. Therefore, to exclude the potential artificial formation of 3-NT by NO_2^-, plasma NO_2^- and NO_3^- concentrations were analyzed using the Griess reaction-based flow reactor system. Both NO_2^- and NO_3^- concentrations did not significantly correlate to 3-NT levels, indicating the exclusion of artificial 3-NT formation in the plasma (data not shown).

Correlation between 3-NT levels as well as prognosis and clinical parameters

To examine the clinical significance of 3-NT levels, we divided the data of patients with ARDS into two groups according to clinical parameters, and the 3-NT levels were compared. We found that plasma 3-NT levels in the non-survivors (median: 0.24, IQR: 0.19-0.36) was lower than survivors (median: 0.43, IQR: 0.11-0.79) (Figure 5A). Similarly, plasma 3-NT levels in ARDS patients without MOF (median: 0.38, IQR: 0.13-0.94) are more likely higher than those with MOF (median: 0.24, IQR: 0.17-0.64). However, the differences were not statistically significant (p = 0.474, 0.235, respectively, Figure 5B). Interestingly, the comparison of the survival curves of ARDS patients who were divided into groups (those with a 3-NT level higher and lower than the mean 3-NT level [0.6 μmol/mol]) revealed that the survival rate was more likely higher in the high 3-NT level group (n = 13) than in the low 3-NT level group (n = 34). However, the difference was not significant (p = 0.232, Log-Rank test, Figure 5C).

Discussion

3-NT is produced by the nitration of tyrosine residues of proteins by RNS such as $ONOO^-$ and NO_2 produced by the reaction of NO and ROS[12,17], or NO_2 produced from NO_2^- by neutrophil myeloperoxidase[16]. 3-NT is used as a biomarker of oxidative stress in numerous infectious and inflammatory conditions, such as ARDS[15]. HPLC-ECD is a quantitative and specific detection method independent of antibodies[20]. Although the use of HPLC-ECD has been reported in animal experiments, reports about its efficiency in detecting 3-NT in human plasma proteins are extremely limited. In this study, we precipitated plasma proteins with ethanol. As a result, the infectious virus particles potentially contained in the plasma could be completely inactivated, which enable a safe transport by air (from Vietnam to Japan). Moreover, NO_2^-, which is the cause of artificial 3-NT formation[22], and the low molecular weight contaminants, which inhibited the performance of HPLC-ECD analysis, were successfully removed. Consequently, 3-NT was quantified with high sensitivity and excellent S/N ratio. In this study, high levels (≥ 1 μmol/mol) of 3-NT were observed in 8 patients, of which 6 survived and 2 died (Figure 2). Plasma 3-NT levels in the survivors was higher than non-survivors, and the survival rate was higher in the high 3-NT level group than in the low

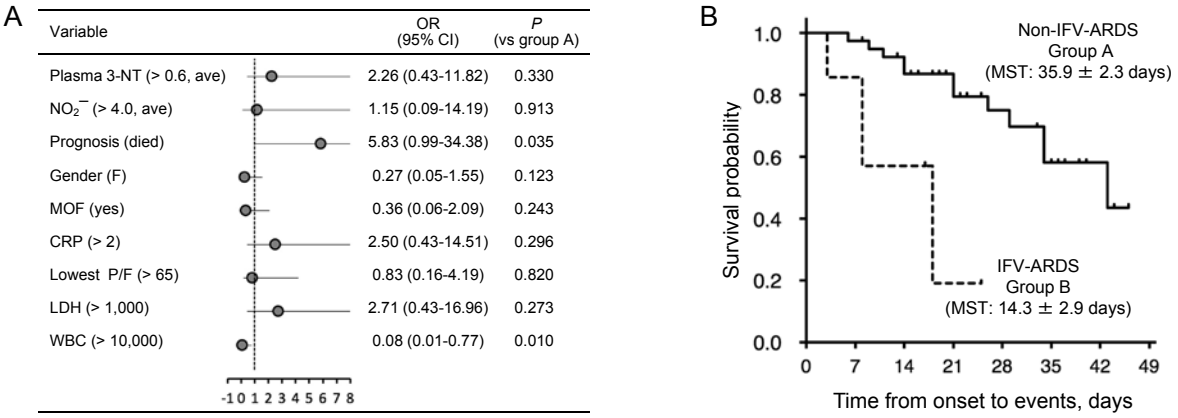

Figure 4. Comparison of Groups A and B via logistic regression analysis and Kaplan Meyer's plot
Compared with non-IFV-ARDS group (group A), IFV-ARDS group (group B) had a higher 3-NT (OR: 2.26). The risk of mortality in group B was 5.83, which indicate a significantly worse prognosis than that in group A. Kaplan Meyer's plot showed significantly lower survival rate of the group B than that of the group A.

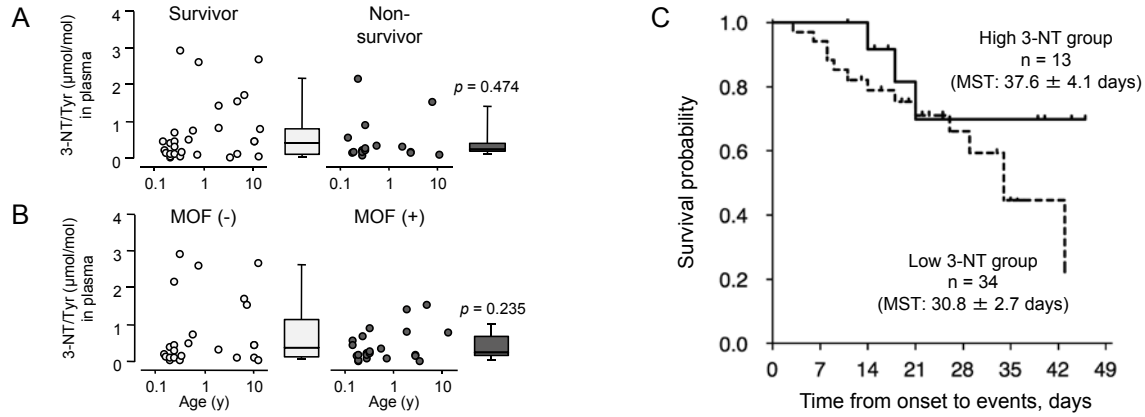

Figure 5. 3-NT levels and prognosis in ARDS patients
3-NT levels in the non-survivors was lower than survivors (A). Similarly, 3-NT levels in ARDS patients without MOF are more likely higher than those with MOF (B). The survival rate was higher in the high 3-NT level group than in the low 3-NT level group (C).

3-NT level group (Figure 5). These results indicates that 3-NT level is not necessarily an indicator of poor prognosis. Rather, the 3-NT level is more likely inversely correlated with prognosis and disease state. The results suggested that the in vivo production of 3-NT by NO/ROS has a direct or indirect biological defense function. NO and ROS are harmful substances that cause pulmonary cell injury and extracellular matrix destruction in ARDS, and they had been considered important effector molecules in ARDS. However, in recent years, NO and ROS have various physiological functions in various cells. We previously found that cGMP, the second messenger of NO signaling, was nitrated to form its unique nitrated derivative 8-nitroguanosine 3', 5'-cyclic monophosphate (8-nitro-cGMP) in cells that depended on the production of NO/ROS[23]. The formation of protein Cys-cGMP adducts by 8-nitro-cGMP was identified as a new post-translational modification, which referred to as protein S-guanylation[23]. Importantly, 8-nitro-cGMP strongly induced antioxidant enzyme heme oxygenase-1 (HO-1) in cultured cells and experimental animal models, indicating the potent signaling functions of 8-nitro-cGMP for HO-1 induction[24,25]. Guanine nitration forming 8-nitro-cGMP may be involved in a unique signal transduction, which contributes to the oxidative stress responses during IFV infection and ARDS[14]. Furthermore, 8-nitro-cGMP and a variety of unidentified nitrated biomolecules may be simultaneously produced in the inflammation site where 3-NT is generated. To understand the entirety of such biological reactions, more studies and case analyses must be assessed. In the future, 3-NT may be further clinically used as a biomarker of oxidative stress in various infectious and inflammatory conditions, such as severe pneumonia and ARDS.

Conflict of interest statement

The authors declare that they have no conflict of interests.

Acknowledgements

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PEER-REVIEWED ORIGINAL ARTICLE

Administration of recombinant single chain fragment of variable region (hScFv) of IgG suppresses development of murine vasculitis induced with *Candida albicans* water-soluble fraction: An animal model of Kawasaki disease

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Abbreviations

ANCA: anti-neutrophilic cytoplasmic antibody, CADS: *Candida albicans* derived substances, CAWS: *Candida albicans* water-soluble fraction, HE: hematoxylin and eosin, hScFv: human single chain fragment of variable region, IgG: immunoglobulin G, IVIg; intravenous immunoglobulin, MPO: myeloperoxidase, SCG/Kj: spontaneous crescentic glomerulonephritis-forming/Kinjoh.

Abstract

***Background*: High-dose intravenous immunoglobulin (IVIg) treatment has been used for therapy of Kawasaki disease and other diseases. Due to the risks of immunoglobulin preparations such as undetectable infection included in donated blood and unknown mechanisms, recombinant immunoglobulins are required. *Candida albicans* water-soluble fraction (CAWS)-induced vasculitis, one of the murine model of Kawasaki disease vasculitis is thought to be suitable for examining the therapeutic effect of recombinant immunoglobulins, because IVIg treatment to CAWS-induced vasculitis by human immunoglobulin was effective. In the present study, we performed histological investigation of inhibitory effect of the recombinant single chain fragment of variable region (hScFv) of IgG on murine model of Kawasaki disease vasculitis.**

***Methods*: The incidence of panvasculitis and histological severity (i.e., the extent of the lesion and the degree of inflammation) of vasculitis were compared among each experimental group in CAWS-induced vasculitis in C57BL/6 mice. The following experimental groups were employed: No treatment (only CAWS injection), solvent, hScFv 2.25 mg/Kg/day, hScFv 4.5 mg/Kg/day, hScFv 9 mg/Kg/day, human native IgG 400 mg/Kg/day.**

***Results:* The incidence of panvasculitis showed in each group as follows. No treatment: 66.7% (4/6), solvent: 40% (2/5), hScFv 2.25 mg/Kg/day: 60% (3/5), hScFv 4.5 mg/Kg/day: 25% (1/4), hScFv 9 mg/Kg/day: 0% (0/1), and native human IgG 400 mg/Kg/day: 40% (2/5), respectively. Panvasculitis was developed in all treated groups other than hScFv 9 mg/kg/day, however the incidence of groups treated with hScFv 4.5 mg/Kg/day and native IgG 400 mg/Kg/day tended to be slightly lower than no treatment group. The extent of the lesion showed in each group as follows. No treatment: 2.33 ± 2.07, solvent: 1.80 ± 2.17, hScFv 2.25 mg/Kg/day: 1.20 ± 1.30, hScFv 4.5 mg/Kg/day: 1.00 ± 1.41, hScFv 9 mg/Kg/day: 1.00, and native IgG 400 mg/Kg/day: 1.80 ± 1.30, respectively. The degree of inflammation showed in each group as follows: No treatment: 6.17 ± 6.52, solvent: 4.80 ± 6.61, hScFv 2.25 mg/Kg/day: 3.60 ± 3.91, hScFv 4.5 mg/Kg/day: 2.00 ± 2.83, hScFv 9 mg/Kg/day: 1.00, and native IgG 400 mg/Kg/day: 4.00 ± 4.18, respectively. There was no significant inter-group variation, the extent of the lesion and degree of inflammation in each treatment group tended to be smaller and milder than those of no treatment group.**

***Conclusion*: The present study suggests that the hScFv has a slightly suppressive effect on development of vasculitis in animal model of Kawasaki disease vasculitis.**

Keywords: Kawasaki disease, hScFv of IgG, Vasculitis, Coronary arteritis, Candida

Introduction

Kawasaki disease is an acute febrile disease of children with unknown cause included in the vasculitis syndrome. Coronary arteries are frequently involved and the formation of coronary artery aneurysms caused by coronary arteritis is the leading cause of ischemic heart disease in children and has a significant impact on the prognosis of the affected child[1]. It is observed that severe inflammatory cell infiltration mainly consisting of macrophages and neutrophils associated with proliferative changes of fibroblasts and capillaries at the site of vasculitis[2-5]. For the first choice in the therapy, high-dose intravenous immunoglobulin (IVIg) treatment has been used[6,7]. Thus, the 24th National Survey of Kawasaki Disease in Japan revealed that 93.5% of patients have been treated with IVIg therapy, showing that efficacy is widely known[8]. However, the mechanism of the inhibitory effect of the IVIg therapy on the vasculitis is still unknown.

Immunoglobulin preparations for IVIg are used not only for Kawasaki disease but also for severe infections and various autoimmune diseases. Although domestic supply of immunoglobulin preparations is secured, the demand is increasing year by year. In addition, immunoglobulin preparations derived from donated blood of healthy volunteers may have a risk for unknown infections. Furthermore, the preparation of immunoglobulins is expensive. Under such circumstances, recombinant immunoglobulins are expected. Kameoka et al[9]. attempted recombinant immunoglobulins, in which the polyclonal mix batch of recombinant single chain fragment of variable region (hScFv) of IgG having VH-CH1 hinge composition was applied to a model mice SCG/Kj for spontaneous development of crescentic glomerulonephritis. The mouse shows an anti-neutrophilic cytoplasmic antibody (ANCA)-related vasculitis. It has been reported that hScFv has a suppressive effect on development and production of myeloperoxidase (MPO)-ANCA, on the other hand human native IgG has slightly suppressive effect on crescentic formation, production of MPO-ANCA[9,10]. However, inhibitory effect of hScFv on other vasculitis model have not been investigated.

A murine model of systemic vasculitis by using *Candida albicans (C. albicans)*-derived substances (CADS) has been established in 1979 by Murata *et al.*[11]. CADS was prepared by alkali extraction of *C. albicans* isolated from the stool of the patients with Kawasaki disease. Later, Ohno clarified that a *C. albicans* water-soluble fraction (CAWS) eluting in the culture supernatant of *C. albicans* grown in a fully synthetic medium also induces vasculitis similar to that in Murata's model[12].

In these models, the lesion distribution and histological images of the vasculitis are similar to those of the vasculitis in Kawasaki disease. The aortic root and the bifurcation of coronary artery are frequently involved by severe inflammatory infiltration mainly consist of neutrophils and macrophages[11-13]. Moreover, the model is thought to be suitable for examining the therapeutic effect of recombinant immunoglobulins, because IVIg treatment to CAWS-induced vasculitis by human immunoglobulin is effective[14].

In the present study, we performed histological investigation of inhibitory effect of hScFv which has been developed by Kameoka *et al*.[9] to the CAWS-induced vasculitis.

Materials and methods

***Animals*:** Mice, C57BL/6, male, 4 weeks of age purchased from Japan SLC, Inc. were used. All animal experiments were implemented following the guidelines from the University of Tokyo Pharmacy and Life Sciences (YAKU 10-47).

***Induction of vasculitis*:** CAWS were used for vasculitis inducer according to the previous report[12,13]. One mg of CAWS suspension in 0.2 mL of PBS and injected intraperitoneally into mouse in a day for 5 consecutive days. The mice were sacrificed 28 days after completion of the continuous inoculation of CAWS under dry ice.

***Preparation of hScFv*:** The polyclonal ScFv antibody mixture was prepared as described elsewhere[9].

***Administration of hScFv for treatment*:** hScFv was intraperitoneally administered for 5 consecutive days after the end of continuous inoculation of CAWS. The dosage of hScFv was 2.25, 4.5, and 9 mg/Kg/day, respectively. Because in SCG/Kj mice, an anti-inflammatory effect of hScFv at a concentration of 20-40 mg/Kg/day has been confirmed[9]. In order to examine the difference in therapeutic effect between hScFv and human immunoglobulin, human native IgG (Nihon Pharmacy Company, Osaka, Japan) was intraperitoneally administered at 400 mg/Kg/day for 5 consecutive days. This dose has been validated for the treatment of Kawasaki disease.

***Experimental group*:** Five groups shown below were set. 1) No treatment (n = 6), 2) Solvent (n = 5), 3) hScFv, 2.25 mg/Kg/day (n = 5), 4) hScFv 4.5 mg/Kg/day (n = 5) , 5) hScFv 9 mg/Kg/day (n = 5), 6) native IgG, 400 mg/Kg/day (n = 5).

***Histological evaluation of vasculitis*:** Histological assessments were carried out in accordance with the previously described methods[13,14]. After the mice were sacrificed, serial sections of the coronary arteries and the aortic root were stained by the hematoxylin and eosin (HE), elastica van Gieson (EvG), and Azan Mallory (AM) staining methods. The stained specimens were carefully examined for inflammatory lesions of the vessel wall under a light microscope. The same site was anatomically divided into 5 segments, i.e., left coronary artery, right coronary artery, left coronary sinus, right coronary sinus, and non-coronary sinus. The degree of inflammation in each segment was assessed using four scores: 0=no inflammation, 1=inflammation in the intima (i.e., endoarteritis), 2=inflammation in the intima and adventitia, and 3=inflammation in all layers of the vascular wall (i.e., panvasculitis) in Table 1. The total number of segments with score 1 or greater was defined as the extent of the lesion, while the total score for all 5 segments was defined as the degree of inflammation for one mouse. Panvasculitis was defined as a positive finding for vasculitis. Comparative investigation was performed regarding the incidence of vasculitis at the coronary artery and aortic root. In order to evaluate the histological severity in the coronary arteries and aortic root, the extent of the lesions and the degree of inflammation were compared among experimental groups.

Table 1. The degree of inflammation in each segment

Score	Evidences	Remarks
0	no inflammation	
1	inflammation in the intima	Endoarteritis
2	inflammation in the intima and adventitia	
3	inflammation in all layers of the vascular wall	Panvasculitis

***Statistical Analysis*:** The incidence of panvasculitis in each group was examined using the chi-square test. The extent of the lesion and the degree of inflammation in each group were analyzed with the Kruskal-Wallis test. In the case that the Kruscal-Wallis test found a significant intergroup variation, the Steel Dwas test was used to perform multiple comparison between experimental groups. However it was impossible to compare between hScFv 9 mg/Kg/day and other groups, because only one mouse was able to survive with hScFv 9 mg/Kg/day. For all tests, $p<0.05$ was defined as representing a significant difference.

Results

***Mouse mortality rate during experiment*:** The mouse mortality rate of each group was hScFv 9 mg/Kg/day: 80% (4/5), and hScFv 4.5 mg/Kg/day: 20% (1/5). However it was difficult to determine the cause of death because no histological analysis was performed on the dead mice in this study.

***Incidence of vasculitis*:** The incidence of panvasculitis in each group was 66.7% (4/6) in no treatment, 40% (2/5) in solvent, 60% (3/5) in treatment with hScFv 2.25 mg/Kg/day, 25% (1/4) in treatment with hScFv 4.5 mg, 0% (0/1) in treatment with hScFv 9 mg/Kg/day, and 40% (2/5) in treatment with native IgG 400 mg/Kg/day (Figure 1). Only one mouse was able to survive with hScFv 9 mg/Kg/day and could not be compared with another mouse. Groups in hScFv 4.5 mg/Kg/day and native IgG 400 mg/Kg/day showed lower incidence than no treatment (p=0.26 and p=0.39, respectively) group. In addition, hScFv 4.5 mg/Kg/day group showed lower incidence than native IgG 400 mg/Kg/day (p=0.16).

Histology of vascular lesions:

-Panvasculitis: Figure 2 shows histological observations of vascular lesions. In no treatment group, panvasculitis was observed in four mice and endoarteritis in the aortic root was observed in other two mice. In two out of four mice with panvasculitis, large lesions that encompassed the aortic root and bilateral coronary arteries were conspicuous (Figure 2 a-d). The other two mice had panvasculitis in only non-coronary sinus. As inflammatory cells neutrophils and macrophages mainly located in the vascular lesion and associated with proliferation of fibroblasts and capillaries. In addition, internal and/or external elastic lamina were destroyed by inflammation and resulting in dilation of blood vessel. Mouse treated with hScFv at 9 mg/Kg/day, minute focus of endoarteritis was observed in aortic root, but panvasculitis defined as inflammation involving all layers of vascular wall was not observed (Figure 2 q, r). Mouse treated with hScFv at 2.25 mg/Kg/day and 4.5 mg/Kg/day, and native IgG 400 mg/Kg/day, panvasculitis occurred but was confined to the aortic root, showing no lesion was found in the coronary artery (Figure 2 i-l, s-v). In addition to this, minute endoarteritis was observed in several places.

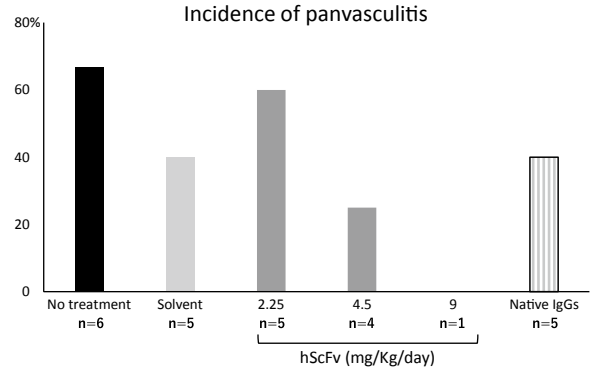

Figure 1. The incidence of panvasculitis
The bar graph shows the incidence of panvasculitis. The black bar: No treatment, light gray bar: solvent, dark gray bars: treatment with hScFv, and stripe bar: treatment with native IgG 400 mg/Kg/day.

-Extent of the lesion: The extent of the lesion (i.e., the total number of involved segments) showed in each group as follows. No treatment: 2.33 ± 2.07, solvent: 1.80 ± 2.17, hScFv 2.25 mg/Kg/day: 1.20 ± 1.30, hScFv 4.5 mg/Kg/day: 1.00 ± 1.41, hScFv 9 mg/Kg/day: 1.00, and native IgG 400 mg/Kg/day: 1.80 ± 1.30, respectively. There was no significant inter-group variation (p=0.71), but the extent of the leion in each treatment group tended to be smaller than no treatment. Also, the extent of the lesion in the hScFv treatment group tended to be smaller than native IgG 400 mg/Kg/day (Figure 3a).

-Degree of inflammation: The degree of inflammation (i.e., the total score for all 5 segments) showed in each group as follows: No treatment: 6.17 ± 6.52, solvent: 4.80 ± 6.61, hScFv 2.25 mg/Kg/day: 3.60 ± 3.91, hScFv 4.5 mg/Kg/day: 2.00 ± 2.83, hScFv 9 mg/Kg/day: 1.00, and native IgG 400 mg/Kg/day: 4.00 ± 4.18 (Figure 3b). There was no significant inter-group variation (p=0.66), and the degree of inflammation for each treatment group tended to be lower than no treatment group. Also, the degree of inflammation in the group of hScFv 4.5 mg/Kg/day tended to be lower than native IgG 400 mg/Kg/day (Figure 3b).

Discussion

In the present study, the inhibitory effects of hScFv on the Kawasaki disease vasculitis model was analyzed histologically. As shown in Figure 1 and 3, the incidence and severity of vasculitis in hScFv 4.5 mg/Kg/day tended to be lower and milder than those in no treatment. hScFv protein derived from a mixture of 204 clones was slightly effective against CAWS-induced vasculitis in murine model of Kawasaki disease vasculitis, suggesting that selected single or a few clone(s) from the 204 clone library may have a higher suppression of the vasculitis. If single or several clones will be selected from 204 clones, it may show high therapeutic effect. Also, hScFv treatment slightly inhibited the development of severity in the CAWS-induced vasculitis in a dose dependent manner, and that was not inferior to native IgG 400 mg/Kg/day. This suggests that hScFv may contain proteins in the clones binding to several key molecules to develop vasculitis, which is considered to be the reason why it is effective at a smaller dose than native human IgG. In the present study, no coronary arteritis was observed in the groups with hScFv. However it could not be clarified that inhibitory effect on coronary arteritis was higher than that of vasculitis in aortic root because the incidence of CAWS-induced coronary arteritis is slightly lower than that of vasculitis in aortic root in both non-treated and treated groups.

Some mice treated with hScFv 9 mg/Kg/day and hScFv 4.5 mg/Kg/day died before the sacrifice. It has been reported that anaphylactic shock, thromboembolism due to an increasing of blood viscosity, and liver injury, and renal disorder are an adverse effect of IVIg therapy. It is very important to determine the cause of death, however it was difficult to determine the cause of death because no histological analysis was performed on the dead mice in this study. Also, for IVIg, high-dose administration of gammaglobulin and its influence on the function of the kidney is not small, therefore the lowest dose showing therapeutic efficacy is important. If it is possible to reduce the dose by using a specific clone with higher vasculitis-suppressing effect, it will also lead to the avoidance of serious adverse events.

This model has been demonstrated that some molecules such as MPO[16] and tumor necrosis factor-α[17] associated with the onset of vasculitis in this model. In addition, mannan, one of the major components of CAWS is essential for development of CAWS-induced vasculitis[18]. Moreover, it may be possible to elucidate the etiology or pathophysiology of vasculitis if an epitope site and target molecule of hScFv is determined. The selected hScFv is expected not only as a therapeutic agent but also as a useful tool for elucidating the etiology and pathophysiology of CAWS-induced vasculitis.

In view of past nation-wide epidemics, it has been suspected that some kind of microorganism is involved in the onset of Kawasaki disease[19], however there is still no consensus regarding the etiology of Kawasaki disease. Regarding the pathophysiology, it was supported that innate immunity affected the onset of Kawasaki disease[20,21]. Recently, we reported that dectin 2, one of the innate immune receptor, was essential for onset of CAWS-induced vasculitis[22]. In this manner, CAWS-induced vasculitis shares many similarities such as not only distribution and histological features of vasculitis but also mechanism of development of vasculitis, in which involvement of microorganisms and the innate immune system is suspected. If the proteins derived from selected clones with high therapeutic effect can be identified, hScFv will be useful for the treatment of Kawasaki disease vasculitis as well as CAWS-induced vasculitis.

Conclusion

The present study suggests that the mix batch of recombinant hScFv proteins derived from 204 clones having VH-CH1 hinge composition suppressed development of vasculitis in animal model of Kawasaki disease vasculitis.

Acknowledgements

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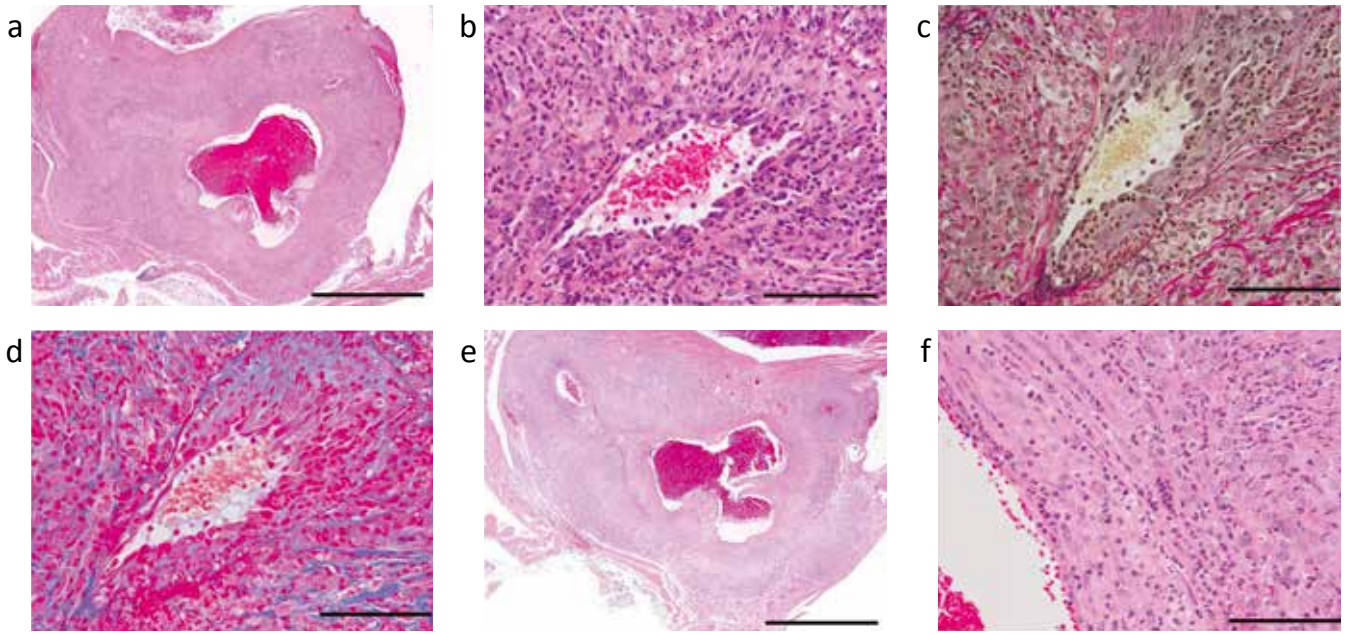
Conflicts of interest

The authors have no conflicts of interest regarding the data reported herein.



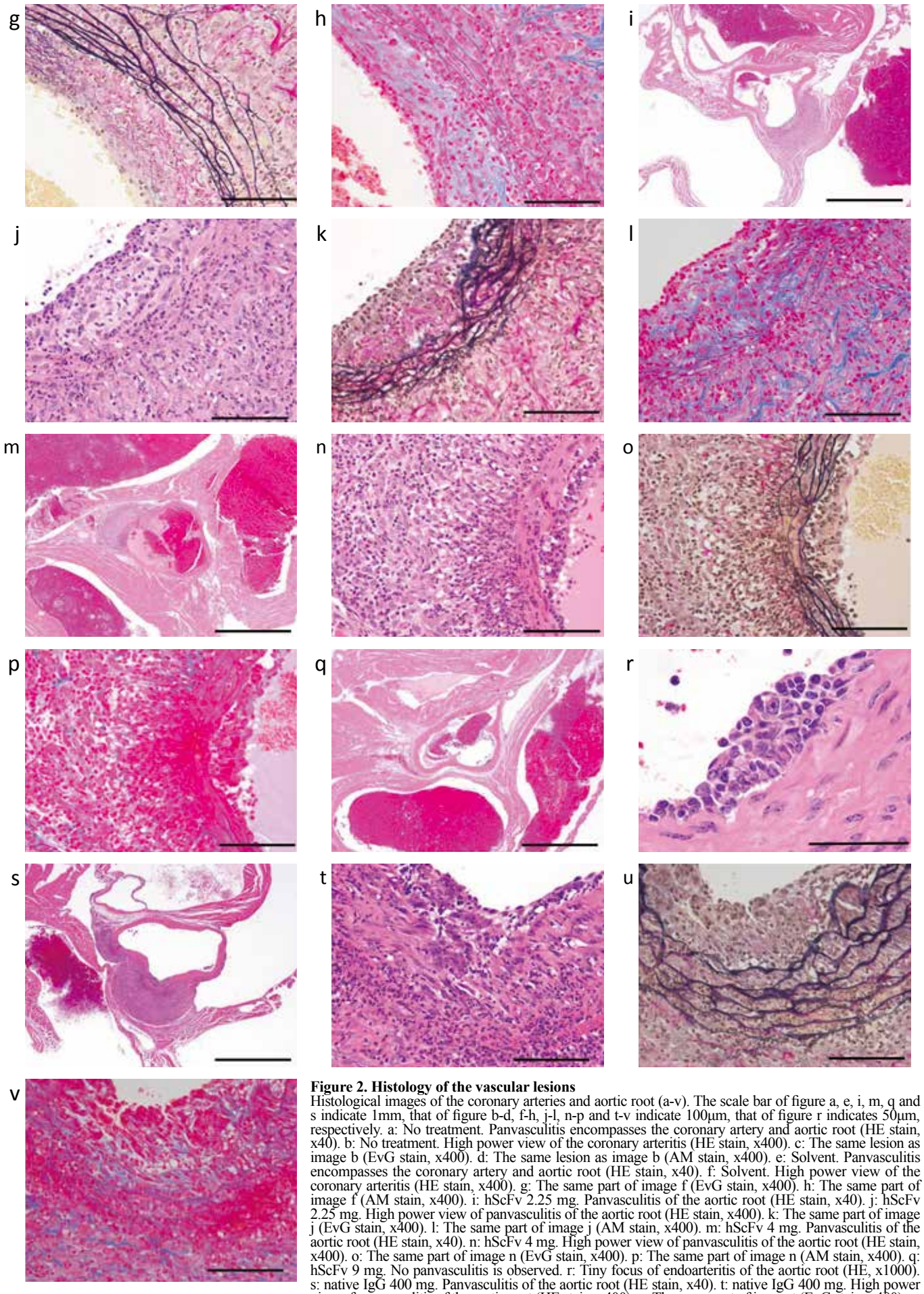


Figure 2. Histology of the vascular lesions
Histological images of the coronary arteries and aortic root (a-v). The scale bar of figure a, e, i, m, q and s indicate 1mm, that of figure b-d, f-h, j-l, n-p and t-v indicate 100μm, that of figure r indicates 50μm, respectively. a: No treatment. Panvasculitis encompasses the coronary artery and aortic root (HE stain, x40). b: No treatment. High power view of the coronary arteritis (HE stain, x400). c: The same lesion as image b (EvG stain, x400). d: The same lesion as image b (AM stain, x400). e: Solvent. Panvasculitis encompasses the coronary artery and aortic root (HE stain, x40). f: Solvent. High power view of the coronary arteritis (HE stain, x400). g: The same part of image f (EvG stain, x400). h: The same part of image f (AM stain, x400). i: hScFv 2.25 mg. Panvasculitis of the aortic root (HE stain, x40). j: hScFv 2.25 mg. High power view of panvasculitis of the aortic root (HE stain, x400). k: The same part of image j (EvG stain, x400). l: The same part of image j (AM stain, x400). m: hScFv 4 mg. Panvasculitis of the aortic root (HE stain, x40). n: hScFv 4 mg. High power view of panvasculitis of the aortic root (HE stain, x400). o: The same part of image n (EvG stain, x400). p: The same part of image n (AM stain, x400). q: hScFv 9 mg. No panvasculitis is observed. r: Tiny focus of endoarteritis of the aortic root (HE, x1000). s: native IgG 400 mg. Panvasculitis of the aortic root (HE stain, x40). t: native IgG 400 mg. High power view of panvasculitis of the aortic root (HE stain, x400). u: The same part of image t (EvG stain, x400). v: The same part of image t (AM stain, x400).

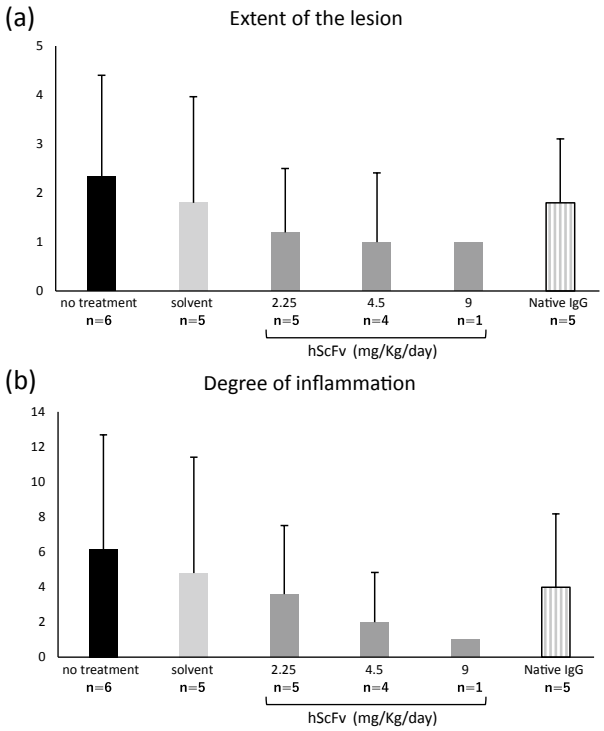


Figure 3. Histological severity of vasculitis
The extent of the lesion (i.e., the total number of involved segments) and (i.e., the total score for all 5 segments) are shown in figure 3a and 3b, respectively. Although there was no intergroup variation, the extent of the lesion and the degree of inflammation all treated groups tended to be smaller and milder than those of no treatment group. The extent of the lesion of all hScFv groups tended to be lower than that of native IgG group. The degree of inflammation of hScFv 4.5 mg/Kg/day tended to be lower than that of native IgG 400 mg/Kg/day.

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Steering Committee Report

March 8, 2019

2018年度　ADC運営委員会記録　日　時：2019年３月８日（金）16時00分 ～ 18時00分

挨拶　沖永佳史 理事長・学長

ADCの役目を年度ごとに確実に進めている。テロや災害、感染症などインターナショナルなリスクが拡大。国の機関と私学のできること、それぞれの特徴を生かし連携を通じで感染症制御ネットワークを構築していければ良いと思う。

議事・討議・審議内容

<2018年度事業報告>　2018年度事業報告の承認：出席22名、委任状19名（運営委員数41名）

1）研究所の現状報告：国費留学生Nguyen Thuy さん2018年９月医学博士取得、附属病院支援、Stem Cell Therapy Consortiumへの協力
2）ADC研究所プログレスレポート
3）国際シンポジウム（ADC設立５周年、他）
4）海外医療機関との研究交流：医学部５年生：ベトナム感染症実習、医学部６年生：海外BSCへ１か月間研修・さくらサイエンスプラン
5）ADC Letter Vol. 5 No. 2, Vol. 6 No. 1　発刊

<2019年度事業計画案>　2019年度事業計画案が承認：出席22名、委任状19名（運営委員数42名）

1）継続事業の計画：病院の支援業務、Stem Cell Therapy Consortium、さくらサイエンスプラン、国際シンポジウム、ADC Letter
2）プロジェクト研究：ブランディング事業は最終年度、新システム「マルチウイルス解析システム」の開発を目指す国費留学生Tran Huu Dat君が学位取得予定（９月）
3）海外医療機関との研究交流　医学部５年生：ベトナム感染症実習・医学部６年生：海外BSC

Research Progress in 2019FY

1. ヒポチオシアンサンによる気道炎症
鈴木章一（准教授）「文科省：科研費基盤（C）代表」

2-1. Development of novel anti-influenza A virus drug based on 16-membered macrolide derivatives
菅又龍一（講師）「文科省：科研費若手（B）代表」

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４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（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および治療法の開発
伊藤吹夕（研究助手）「厚生労働省研究班：研究協力者」「文科省：科研費基盤（C）２課題　代表および分担」

4. 研究ブランディング「グローバルな視点からの危機管理：パンデミック感染症対応のマルチウイルス検出・解析システムの構築」　鈴木和男（教授・所長）、他

INTERNATIONAL MEETING AND SYMPOSIUM

開催したイベント（2019.1.1～2019.6.30）

日程	イベント名	演者など	
2019年5月20日(月)～22日(水)	第2回 Stem Cell Therapy Consortium 国際会議	鈴木和男	Vinmec, Hanoi, Vietnam
2019年5月14日(火)	危機管理と防災	板橋キャンパス危機管理委員会、ADC研	臨床大講堂
2019年5月13日(月)～6月7日(金)	医学部6年生(2名)海外BSC	Cambridge Univ., UK, Joslin Diabetes Center, Boston	
2019年5月7日(火)	第4回 Stem Cell Therapy Consortium会議	長村、鈴木、吉岡、伊藤	東京大学医科学研究所
2019年4月7日(日)～10日(水)	The 19th International Vasculitis and ANCA Workshop/EUVAS-VCRCIM	Peter Merkel, 鈴木和男	Philadelphia PA, United States of America
2019年3月8日(金)	ADC運営委員会		大学棟 会議室
2019年2月23日(土)、4月3日(水)	第2,3回 Stem Cell Therapy Consortium会議		日暮里、大学棟会議室
2019年1月29日(火)	第1回 Stem Cell Therapy Consortium国際会議	鈴木和男、岡崎富男、布井博幸	Vinmec, Hanoi, Vietnam
2019年1月11日(金)	第2回 バイオセキュリティ講習会(日本語)	棚林清 感染研バイオセーフティ管理室 室長	大学棟 講義室

今後のイベント情報（2019.7.1～2019.12.31）

日程	イベント名	演者など	
2019年12月9日(月)～13日(金)	Antibody Drug会議	鈴木和男	San Diego, USA
2019年11月29日(金)～30日(土)	第25回 MPO研究会	ADC研	順天堂大学
2019年11月8日(金)	VCRCIM	鈴木和男	Atlanta, USA
2019年10月30日(水)	第1回 バイオセキュリティ講習会(英語)	棚林清 感染研バイオセーフティ管理室 室長	大学棟
2019年10月28日(月)～11月6日(水)	SAKURA Science Plan 2018	Vietnamから研修生 8名（ハノイ･ホーチミン）	大学棟、附属病院
2019年9月21日(土)～23日(月)	第6回 国際バイオイメージングシンポジウム	ADC研	帝京大学板橋キャンパス
2019年9月4日(水)～7日(土)	11th International Human Peroxidase Meeting	鈴木和男	Brno, Czech
2019年8月30日(金)	TAVP 報告会（ベトナム感染症）	医学部5年生 6名、教員	本部棟
2019年8月27日(火)	第2回 帝京大学研究交流シンポジウム	ADC研	大学棟
2019年8月18日(日)～25日(日)	TAVP Training for 6 Students (5-year)	国立小児病院、ハノイ医科大学ほか	Hanoi, Vietnam
2019年7月31日(水)～8月1日(木)	10周年記念講演会:National Institute of Respiratory Diseases	鈴木和男	Mexico City
2019年7月29日(月)～30日(火)	SCTC共同研究会議	鈴木和男	NIH, USA
2019年7月10日(水)～13日(土)	ベトナム国立小児病院50周年記念シンポジウム	鈴木和男	Hanoi, Vietnam

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