ReDURCクロージング・フォーラム（2024.3.20）

デュアルユースについて考えを深めるには どのようにしたらよいか？

NIID 国立感染症研究所
安全実験管理部
花木　賢一

問い

感染症（・病原体）研究者に
デュアルユース問題
を意識させるにはどうしたらよいか？

バイオセーフティとバイオセキュリティ

バイオリスク管理

バイオセーフティ

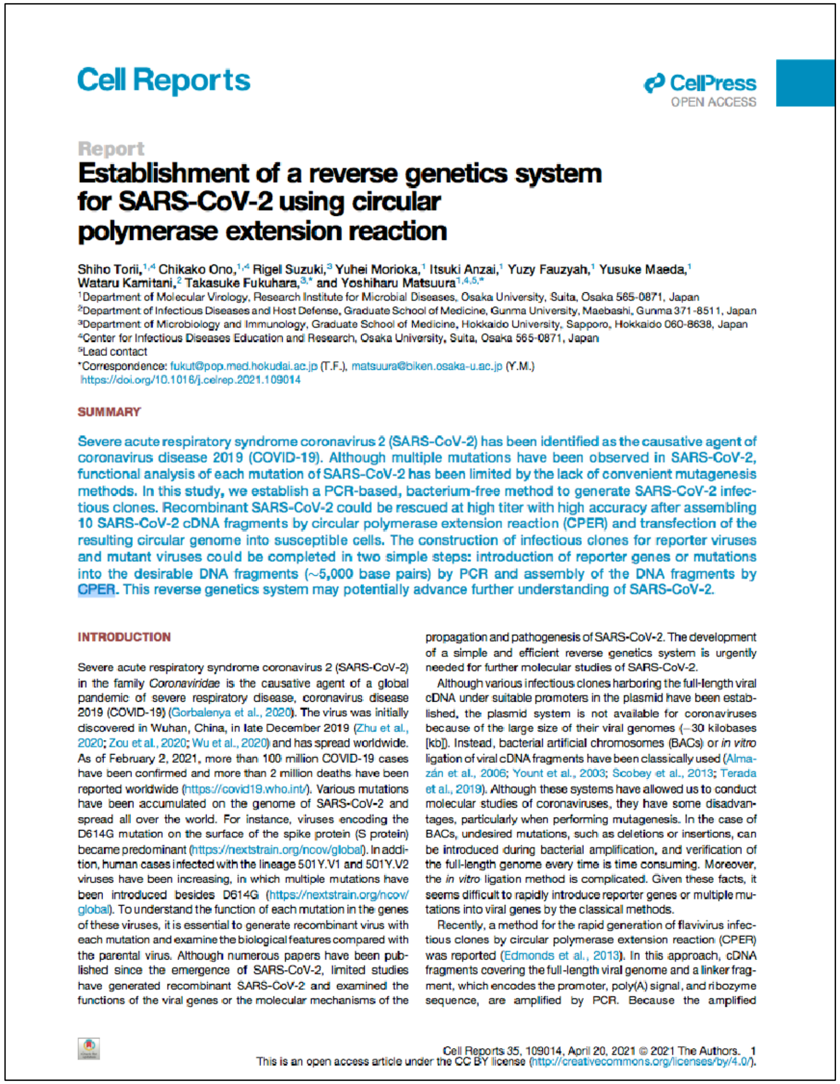
バイオセキュリティ

- **微生物実験手技**
- **標準作業手順**（SOP）
- **一次封じ込め**（安全キャビネット、個人防護具等）
- **二次封じ込め**（施設、空調等）
- **教育訓練**

- アクセス制御
- 人事管理
- 病原体等の管理
- 廃棄物の適切な除染/処分
- 適切な輸送手順
- 災害、防犯等訓練

- ロック付きドア
- パスワード/ PIN
- カードリーダー
- 生体認証（指紋）
- 監視カメラ
- 情報セキュリティ
- 警備員
- フェンス
- 窓の格子
- 磁気ロック
- ドアの磁気スイッチ
- 警報装置

日本発のデュアルユース性がある研究例



Cell Reports

CellPress
OPEN ACCESS

Report

Establishment of a reverse genetics system for SARS-CoV-2 using circular polymerase extension reaction

Shiho Torii,[1,4] Chikako Ono,[1,4] Rigel Suzuki,[3] Yuhei Morioka,[1] Itsuki Anzai,[1] Yuzy Fauzyah,[1] Yusuke Maeda,[1] Wataru Kamitani,[2] Takasuke Fukuhara,[3,*] and Yoshiharu Matsuura[1,4,5,*]
[1]Department of Molecular Virology, Research Institute for Microbial Diseases, Osaka University, Suita, Osaka 565-0871, Japan
[2]Department of Infectious Diseases and Host Defense, Graduate School of Medicine, Gunma University, Maebashi, Gunma 371-8511, Japan
[3]Department of Microbiology and Immunology, Graduate School of Medicine, Hokkaido University, Sapporo, Hokkaido 060-8638, Japan
[4]Center for Infectious Diseases Education and Research, Osaka University, Suita, Osaka 565-0871, Japan
[5]Lead contact
*Correspondence: fukut@pop.med.hokudai.ac.jp (T.F.), matsuura@biken.osaka-u.ac.jp (Y.M.)
https://doi.org/10.1016/j.celrep.2021.109014

SUMMARY

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been identified as the causative agent of coronavirus disease 2019 (COVID-19). Although multiple mutations have been observed in SARS-CoV-2, functional analysis of each mutation of SARS-CoV-2 has been limited by the lack of convenient mutagenesis methods. In this study, we establish a PCR-based, bacterium-free method to generate SARS-CoV-2 infectious clones. Recombinant SARS-CoV-2 could be rescued at high titer with high accuracy after assembling 10 SARS-CoV-2 cDNA fragments by circular polymerase extension reaction (CPER) and transfection of the resulting circular genome into susceptible cells. The construction of infectious clones for reporter viruses and mutant viruses could be completed in two simple steps: introduction of reporter genes or mutations into the desirable DNA fragments (~5,000 base pairs) by PCR and assembly of the DNA fragments by CPER. This reverse genetics system may potentially advance further understanding of SARS-CoV-2.

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in the family *Coronaviridae* is the causative agent of a global pandemic of severe respiratory disease, coronavirus disease 2019 (COVID-19) (Gorbalenya et al., 2020). The virus was initially discovered in Wuhan, China, in late December 2019 (Zhu et al., 2020; Zou et al., 2020; Wu et al., 2020) and has spread worldwide. As of February 2, 2021, more than 100 million COVID-19 cases have been confirmed and more than 2 million deaths have been reported worldwide (https://covid19.who.int/). Various mutations have been accumulated on the genome of SARS-CoV-2 and spread all over the world. For instance, viruses encoding the D614G mutation on the surface of the spike protein (S protein) became predominant (https://nextstrain.org/ncov/global). In addition, human cases infected with the lineage 501Y.V1 and 501Y.V2 viruses have been increasing, in which multiple mutations have been introduced besides D614G (https://nextstrain.org/ncov/global). To understand the function of each mutation in the genes of these viruses, it is essential to generate recombinant virus with each mutation and examine the biological features compared with the parental virus. Although numerous papers have been published since the emergence of SARS-CoV-2, limited studies have generated recombinant SARS-CoV-2 and examined the functions of the viral genes or the molecular mechanisms of the propagation and pathogenesis of SARS-CoV-2. The development of a simple and efficient reverse genetics system is urgently needed for further molecular studies of SARS-CoV-2.

Although various infectious clones harboring the full-length viral cDNA under suitable promoters in the plasmid have been established, the plasmid system is not available for coronaviruses because of the large size of their viral genomes (~30 kilobases [kb]). Instead, bacterial artificial chromosomes (BACs) or *in vitro* ligation of viral cDNA fragments have been classically used (Almazán et al., 2006; Yount et al., 2003; Scobey et al., 2013; Terada et al., 2019). Although these systems have allowed us to conduct molecular studies of coronaviruses, they have some disadvantages, particularly when performing mutagenesis. In the case of BACs, undesired mutations, such as deletions or insertions, can be introduced during bacterial amplification, and verification of the full-length genome every time is time consuming. Moreover, the *in vitro* ligation method is complicated. Given these facts, it seems difficult to rapidly introduce reporter genes or multiple mutations into viral genes by the classical methods.

Recently, a method for the rapid generation of flavivirus infectious clones by circular polymerase extension reaction (CPER) was reported (Edmonds et al., 2013). In this approach, cDNA fragments covering the full-length viral genome and a linker fragment, which encodes the promoter, poly(A) signal, and ribozyme sequence, are amplified by PCR. Because the amplified

Cell Reports 35, 109014, April 20, 2021 © 2021 The Authors. 1

（Torii et al., Cell Rep. 35:109014, 2021）

『環状ポリメラーゼ伸長反応を用いたSARS-CoV-2の逆遺伝学システムの構築』

【研究概要】Circular Polymerase Extension Reaction （CPER） 法を用いることで、**新型コロナウイルスを2週間で人工合成する技術を確立**した。

【意義】SARS-CoV-2の人工合成技術を誰もが実施できるように簡単にした。人為的に遺伝子変異を導入したウイルスを短期間で作出でき、**病原性の解析、ワクチンや抗ウイルス薬の開発を加速化**できる。

【リスク】既存の**ワクチンや抗ウイルス薬を無効化する変異株を短期間で創出できる**。悪意をもって**パンデミックを引き起こす新たなコロナウイルスを創出することへ利用される可能性**がある。

日本発のデュアルユース性がある研究例

数ヶ月を2週間に！迅速・簡便な新型コロナウイルス人工合成技術を開発

新型コロナウイルス関連研究の加速化に貢献

2021-4-13 ● 生命科学・医学系
微生物病研究所　教授　松浦善治

目次

本研究の成果

デング熱を起こすデングウイルスなどが含まれるフラビウイルスでは、Circular Polymerase Extension Reaction (CPER) 法というPCRを活用した手法で、感染性ウイルスクローンを作出する技術が開発されています。本研究ではこのCPER法を新型コロナウイルスにも応用できないかと考えて研究を進めました。

まず、新型コロナウイルスの遺伝子全長をカバーする9個のウイルス遺伝子断片とプロモーターを含むリンカー断片をPCRで増幅しました（図のステップ1）。各断片が隣り合う断片と重なる領域を持つよう設計することで、もう一度PCRを行うと、10個の断片が一つに繋がり、ウイルス遺伝子全長をコードする環状のDNAを作製できることがわかりました（図のステップ2）。この環状DNAを新型コロナウイルスがよく増殖する培養細胞に導入すると、細胞の中でDNAをもとにRNAが合成され、さらにこのRNAをもとにウイルスが合成されて、約7日間で感染性の新型コロナウイルスを作出することができました (図のステップ3）。すなわちCPER法を用いることで、高度な遺伝子操作技術を用いずに、PCRのみで新型コロナウイルスの感染性DNAクローンを作製できることが分かりました。さらに、GFPなどの蛍光タンパク質を導入したウイルスや、任意の遺伝子を変異させたウイルスも作出可能であることを示しました。

GFPなどの蛍光タンパク質を導入したウイルス

ウイルスに蛍光タンパク質を導入すると、ウイルスが感染した細胞で蛍光タンパク質が発現するため、感染細胞を可視化することができる。この技術により薬剤の抗ウイルス活性や、ウイルスの感染性を測定可能になる。

本研究成果の意義

本研究は、新型コロナウイルスの性状解析において課題であった人工合成技術を、誰もが実施できるように簡単にした、まさにコロンブスの卵のような研究です。

より多くの研究者が迅速・簡便に新型コロナウイルスを合成できるようになることで、人工的に遺伝子改変したウイルスを用いた病原性解析やワクチン・抗ウイルス薬の開発、また、次々と現れる変異ウイルスに対するこれまで以上に素早い解析が可能となり、新型コロナウイルス感染症克服に向けた研究が飛躍的に進むことが期待されます。

コロンブスの卵

アメリカ大陸発見は誰にでもできると言われたコロンブスが、卵を立てることを試みさせ、誰もできなかった後に卵の先端を潰して立てて見せたという逸話から、誰もが思いつきそうな簡単なことでも、それを最初に行うことは難しいことの例え。また盲点のことを言う。

特記事項

【掲載論文】“Establishment of a reverse genetics system for SARS-CoV-2 using circular polymerase extension reaction”
【著者】Shiho Torii, Chikako Ono, Rigel Suzuki, Yuhei Morioka, Itsuki Anzai, Yuzy Fauzyah, Yusuke Maeda, Wataru Kamitani, Takasuke Fukuhara, Yoshiharu Matsuura
【掲載誌】Cell Reportsに、2021年4月にオンライン掲載

本研究は、科学研究費補助金、日本医療研究開発機構 (AMED) 新興・再興感染症に対する革新的医薬品等開発推進研究事業、JST【ムーンショット型研究開発事業】グラント番号【JPMJMS2025】「ウイルス-人体相互作用ネットワークの理解と制御」の支援を得て行われました。

［閲覧元］大阪大学の研究ポータルサイト（https://resou.osaka-u.ac.jp/ja/research/2021/20210413_1）

日本発のデュアルユース性がある研究例

トップ > ニュース > 成果情報 > 数ヶ月を2週間に！迅速・簡便な新型コロナウイルス人工合成技術を開発―新型コロナウイルス関連研究の加速化に貢献―

成果情報

数ヶ月を2週間に！迅速・簡便な新型コロナウイルス人工合成技術を開発―新型コロナウイルス関連研究の加速化に貢献―

本研究成果の意義

本研究は、新型コロナウイルスの性状解析において課題であった人工合成技術を、誰もが実施できるように簡単にした、まさにコロンブスの卵※3のような研究です。

より多くの研究者が迅速・簡便に新型コロナウイルスを合成できるようになることで、人工的に遺伝子改変したウイルスを用いた病原性解析やワクチン・抗ウイルス薬の開発、また、次々と現れる変異ウイルスに対するこれまで以上に素早い解析が可能となり、新型コロナウイルス感染症克服に向けた研究が飛躍的に進むことが期待されます。

特記事項

本研究は、科学研究費補助金、日本医療研究開発機構（AMED）新興・再興感染症に対する革新的医薬品等開発推進研究事業、JST【ムーンショット型研究開発事業】グラント番号【JPMJMS2025】「ウイルス-人体相互作用ネットワークの理解と制御」の支援を得て行われました。

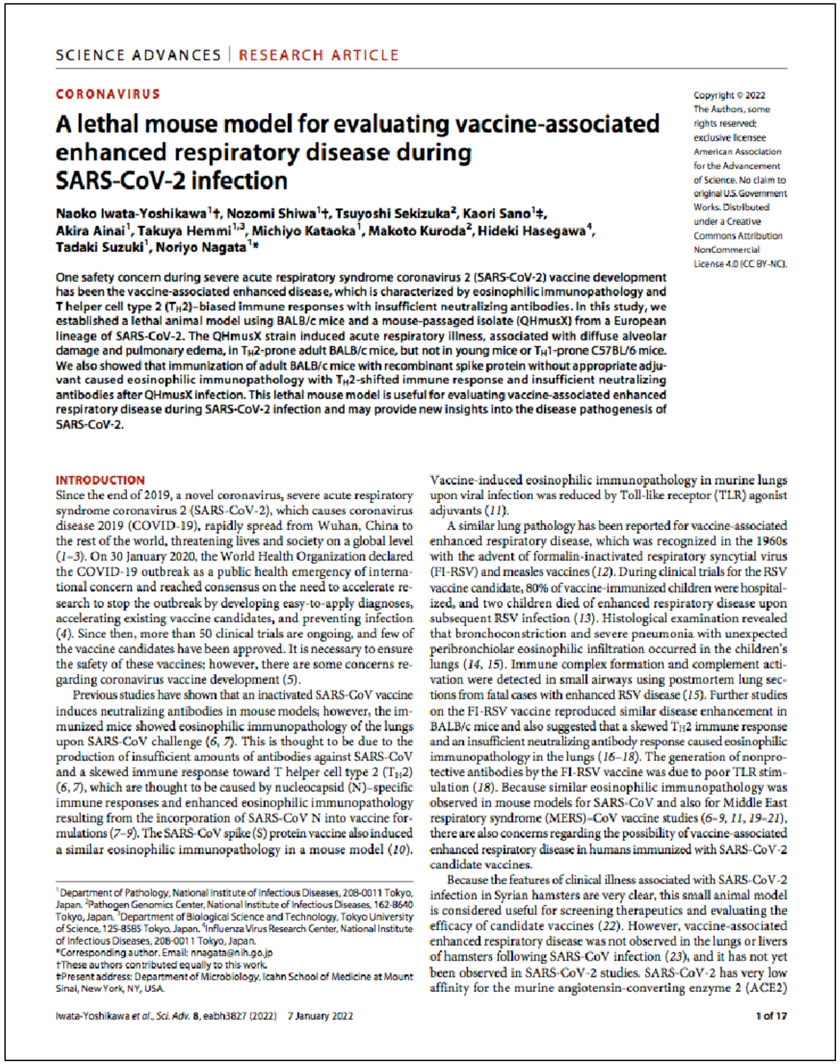
Cell Reportsに、2021年4月にオンライン掲載

特記事項

本研究は、科学研究費補助金、日本医療研究開発機構（AMED）新興・再興感染症に対する革新的医薬品等開発推進研究事業、JST【ムーンショット型研究開発事業】グラント番号【JPMJMS2025】「ウイルス-人体相互作用ネットワークの理解と制御」の支援を得て行われました。

［閲覧元］AMEDニュース > 成果情報（https://www.amed.go.jp/news/seika/kenkyu/20210524.html）

感染研所属研究者による機能獲得研究



SCIENCE ADVANCES | RESEARCH ARTICLE

CORONAVIRUS

A lethal mouse model for evaluating vaccine-associated enhanced respiratory disease during SARS-CoV-2 infection

Naoko Iwata-Yoshikawa[1]†, Nozomi Shiwa[1]†, Tsuyoshi Sekizuka[2], Kaori Sano[1]‡, Akira Ainai[1], Takuya Hemmi[1,3], Michiyo Kataoka[1], Makoto Kuroda[2], Hideki Hasegawa[4], Tadaki Suzuki[1], Noriyo Nagata[1]*

One safety concern during severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccine development has been the vaccine-associated enhanced disease, which is characterized by eosinophilic immunopathology and T helper cell type 2 (T_H2)–biased immune responses with insufficient neutralizing antibodies. In this study, we established a lethal animal model using BALB/c mice and a mouse-passaged isolate (QHmusX) from a European lineage of SARS-CoV-2. The QHmusX strain induced acute respiratory illness, associated with diffuse alveolar damage and pulmonary edema, in T_H2-prone adult BALB/c mice, but not in young mice or T_H1-prone C57BL/6 mice. We also showed that immunization of adult BALB/c mice with recombinant spike protein without appropriate adjuvant caused eosinophilic immunopathology with T_H2-shifted immune response and insufficient neutralizing antibodies after QHmusX infection. This lethal mouse model is useful for evaluating vaccine-associated enhanced respiratory disease during SARS-CoV-2 infection and may provide new insights into the disease pathogenesis of SARS-CoV-2.

INTRODUCTION

Since the end of 2019, a novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes coronavirus disease 2019 (COVID-19), rapidly spread from Wuhan, China to the rest of the world, threatening lives and society on a global level (*1*–*3*). On 30 January 2020, the World Health Organization declared the COVID-19 outbreak as a public health emergency of international concern and reached consensus on the need to accelerate research to stop the outbreak by developing easy-to-apply diagnoses, accelerating existing vaccine candidates, and preventing infection (*4*). Since then, more than 50 clinical trials are ongoing, and few of the vaccine candidates have been approved. It is necessary to ensure the safety of these vaccines; however, there are some concerns regarding coronavirus vaccine development (*5*).

Previous studies have shown that an inactivated SARS-CoV vaccine induces neutralizing antibodies in mouse models; however, the immunized mice showed eosinophilic immunopathology of the lungs upon SARS-CoV challenge (*6*, *7*). This is thought to be due to the production of insufficient amounts of antibodies against SARS-CoV and a skewed immune response toward T helper cell type 2 (T_H2) (*6*, *7*), which are thought to be caused by nucleocapsid (N)–specific immune responses and enhanced eosinophilic immunopathology resulting from the incorporation of SARS-CoV N into vaccine formulations (*7*–*9*). The SARS-CoV spike (S) protein vaccine also induced a similar eosinophilic immunopathology in a mouse model (*10*). Vaccine-induced eosinophilic immunopathology in murine lungs upon viral infection was reduced by Toll-like receptor (TLR) agonist adjuvants (*11*).

A similar lung pathology has been reported for vaccine-associated enhanced respiratory disease, which was recognized in the 1960s with the advent of formalin-inactivated respiratory syncytial virus (FI-RSV) and measles vaccines (*12*). During clinical trials for the RSV vaccine candidate, 80% of vaccine-immunized children were hospitalized, and two children died of enhanced respiratory disease upon subsequent RSV infection (*13*). Histological examination revealed that bronchoconstriction and severe pneumonia with unexpected peribronchiolar eosinophilic infiltration occurred in the children's lungs (*14*, *15*). Immune complex formation and complement activation were detected in small airways using postmortem lung sections from fatal cases with enhanced RSV disease (*15*). Further studies on the FI-RSV vaccine reproduced similar disease enhancement in BALB/c mice and also suggested that a skewed T_H2 immune response and an insufficient neutralizing antibody response caused eosinophilic immunopathology in the lungs (*16*–*18*). The generation of nonprotective antibodies by the FI-RSV vaccine was due to poor TLR stimulation (*18*). Because similar eosinophilic immunopathology was observed in mouse models for SARS-CoV and also for Middle East respiratory syndrome (MERS)–CoV vaccine studies (*6*–*9*, *11*, *19*–*21*), there are also concerns regarding the possibility of vaccine-associated enhanced respiratory disease in humans immunized with SARS-CoV-2 candidate vaccines.

Because the features of clinical illness associated with SARS-CoV-2 infection in Syrian hamsters are very clear, this small animal model is considered useful for screening therapeutics and evaluating the efficacy of candidate vaccines (*22*). However, vaccine-associated enhanced respiratory disease was not observed in the lungs or livers of hamsters following SARS-CoV infection (*23*), and it has not yet been observed in SARS-CoV-2 studies. SARS-CoV-2 has very low affinity for the murine angiotensin-converting enzyme 2 (ACE2)

---

[1] Department of Pathology, National Institute of Infectious Diseases, 208-0011 Tokyo, Japan. [2] Pathogen Genomics Center, National Institute of Infectious Diseases, 162-8640 Tokyo, Japan. [3] Department of Biological Science and Technology, Tokyo University of Science, 125-8585 Tokyo, Japan. [4] Influenza Virus Research Center, National Institute of Infectious Diseases, 208-0011 Tokyo, Japan.
*Corresponding author. Email: nnagata@nih.go.jp

†These authors contributed equally to this work.

‡Present address: Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, NY, USA.

Iwata-Yoshikawa *et al.*, *Sci. Adv.* 8, eabh3827 (2022) 7 January 2022 1 of 17

『SARS-CoV-2感染におけるワクチン関連呼吸器疾患亢進を評価するための致死マウスモデル』

【研究概要】BALB/cマウスとSARS-CoV-2のヨーロッパ系統由来マウス継代分離株(QHmusX) を用いた**致死動物モデルを確立**する。

【意義】致死マウスモデルは、SARS-CoV-2の疾患発症機序に関する**新たな知見を提供する**可能性がある。

【方法】マウスに対して非致死的なSARS-CoV-2分離株がマウスに対して致死的なウイルスを作出するために、**若齢マウスを用いて10回連続継代した**。

（Iwata-Yoshikawa et al., Sci Adv. 8:eabh3827, 2022）

天然痘ワクチンを無効化する研究

Journal of Virology, Feb. 2001, p. 1205–1210 Vol. 75, No. 3
0022-538X/01/$04.00+0 DOI: 10.1128/JVI.75.3.1205–1210.2001

Expression of Mouse Interleukin-4 by a Recombinant Ectromelia Virus Suppresses Cytolytic Lymphocyte Responses and Overcomes Genetic Resistance to Mousepox

RONALD J. JACKSON,[1,2]* ALISTAIR J. RAMSAY,[2]† CARINA D. CHRISTENSEN,[2] SANDRA BEATON,[1]
DIANA F. HALL,[1]‡ AND IAN A. RAMSHAW[2]

Pest Animal Control Cooperative Research Centre, CSIRO Sustainable Ecosystems,[1] and Division of Immunology and Cell Biology, John Curtin School of Medical Research, Australian National University,[2] Canberra, Australia

Received 25 July 2000/Accepted 13 November 2000

Genetic resistance to clinical mousepox (ectromelia virus) varies among inbred laboratory mice and is characterized by an effective natural killer (NK) response and the early onset of a strong $CD8^+$ cytotoxic T-lymphocyte (CTL) response in resistant mice. We have investigated the influence of virus-expressed mouse interleukin-4 (IL-4) on the cell-mediated response during infection. It was observed that expression of IL-4 by a thymidine kinase-positive ectromelia virus suppressed cytolytic responses of NK and CTL and the expression of gamma interferon by the latter. Genetically resistant mice infected with the IL-4-expressing virus developed symptoms of acute mousepox accompanied by high mortality, similar to the disease seen when genetically sensitive mice are infected with the virulent Moscow strain. Strikingly, infection of recently immunized genetically resistant mice with the virus expressing IL-4 also resulted in significant mortality due to fulminant mousepox. These data therefore suggest that virus-encoded IL-4 not only suppresses primary antiviral cell-mediated immune responses but also can inhibit the expression of immune memory responses.

Ectromelia virus (ECTV; family *Poxviridae*, genus *Orthopoxvirus*) is a natural pathogen of laboratory mice that causes a generalized disease termed mousepox (13). All mice are equally susceptible to infection by footpad inoculation; however, development of clinical mousepox among inbred mouse strains differs greatly (44). In mousepox-sensitive (e.g., BALB/c) mice, the disease is an acute systemic infection with high viral titers in the liver and spleen with resultant necrosis and high mortality. In contrast, infection of mousepox-resistant (e.g., C57BL/6) mice is usually subclinical, with lower levels of viral replication in the visceral organs and development of nonfatal lesions. Genetic resistance has been found to act through the combined activity of innate host defenses including natural killer (NK) cells, alpha interferon (IFN-α), IFN-β, IFN-γ, activated macrophages, and inducible nitric oxide production (17, 21, 23, 24, 36). Mousepox-resistant mice also display the early activation of a strong virus-specific cytotoxic T-lymphocyte (CTL) response (20, 32) and produce high levels of type 1 cytokines interleukin-2 (IL-2), IL-12, IFN-γ, and tumor necrosis factor alpha (TNF-α) in response to ECTV infection, whereas these factors are absent or produced at low levels in susceptible mice (19, 36).

Effector $CD4^+$ T cells can be categorized on the basis of their cytokine production either as T helper 1 (Th1) cells that produce IL-2 and IFN-γ or T helper 2 (Th2) cells that produce predominantly IL-4, IL-5, IL-10, and IL-13 (40). The cross-regulatory activities of IL-12 and IL-4, factors that play key roles in directing the development of the Th1 and Th2 subsets, respectively, is well characterized (40). Both in vitro and in vivo, the presence of IL-4 at the time of stimulation has been shown to inhibit IL-12 expression by antigen-presenting cells (macrophages and dendritic cells), with Th2 cells dominantly expanded in the acquired response (7, 8, 25). In addition to its effects on development of Th1 and Th2 subsets, IL-4 has been shown to influence the differentiation of other lymphocyte types. In vitro stimulation of naive $CD8^+$ cells in the presence of IL-2, IL-12, or IFN-γ generates classical type 1 cytotoxic cells (Tc1) which express IFN-γ; however, $CD8^+$ cells stimulated in the presence of IL-4 may develop a Tc2 phenotype expressing the cytokines IL-4, IL-5, IL-6, IL-10, sometimes with reduced cytoxicity (11, 38). Treatment of activated Tc1 cells with IL-4 results in defective IFN-γ, TNF-α, and IL-2 expression. Although IL-4-treated Tc1 cells retain short-term in vitro cytotoxic activity, they fail to proliferate in response to antigen stimulation, compromising their long-term functional capability to control infection (37). It has recently been shown that NK cells cultured in the presence of IL-12 or IL-4 may also differentiate into NK1 or NK2 cells, respectively, with distinct patterns of cytokine secretion similar to those of Th1 and Th2 cells, although this does not appear to affect their in vitro cytotoxic activity (33).

Cross-regulation of Th subsets and the generation of an appropriate type of immune response against a particular pathogen is important since the dominance of an inappropriate response can exacerbate disease and lead to the inability to eradicate the infecting organism. The use of recombinant vaccinia virus (VACV) to study the in vivo effects of mouse cytokines has demonstrated that the course of infection can be mediated and biased toward either an antiviral effect by coexpression of type 1 cytokines or enhanced virus virulence by

---

* Corresponding author. Mailing address: CSIRO Sustainable Ecosystems, GPO Box 284, Canberra ACT 2601, Australia. Phone: 61 (02) 6242 1717. Fax: 61 (02) 6242 1511. E-mail: R.Jackson@csc.csiro.au.

† Present address: Centre for Biomolecular Vaccine Technology, Discipline of Immunology and Microbiology, University of Newcastle, Newcastle, New South Wales, Australia.

‡ Present address: CSIRO Plant Industry, Canberra ACT, Australia.

（Jackson et al. J Virol. 75:1205–1210, 2001）

- 研究の背景 -

オーストラリアの外来生物であるアナウサギをポックスウイルスを用いて不妊化させて個体数を減らす研究を行うため、マウスとマウスに感染するポックスウイルスを代用した。

遺伝子組換えポックスウイルスはポックスウイルスに抵抗力のあるマウス系統で、事前に免疫したマウスを死に至らしめた。

ポックスウイルスは天然痘ウイルスに近縁のため、同じ技術を天然痘ウイルスに応用すると・・・。

論文への批判、批評記事

NewScientist

Killer mousepox virus raises bioterror fears

キラーマウスポックスウイルスがバイオテロの恐怖を高める

By Rachel Nowak
10 January 2001

The Guardian

Lab creates killer virus by accident

研究室が殺人ウイルスを偶然に作成

By Tim Radford
11 January 2001

The New York Times

Australians Create a Deadly Mouse Virus

オーストラリア人が致死性のマウスウイルスを作成

By William J. Broad
23 January 2001

天然痘ワクチンを無効化する研究

EMBO *reports*

outlook

The mousepox experience

An interview with Ronald Jackson and Ian Ramshaw on dual-use research

Michael J. Selgelid & Lorna Weir

Much of the debate about science policy in recent years has focused on 'the dual-use dilemma', which arises when well-intentioned scientific research has the potential to be misused by state and non-state actors for nefarious purposes. In the context of the life sciences, for example, the same discoveries that lead to advancements in medicine could also be used to facilitate the development of biological weapons. Although all life science techniques and discoveries might be inherently dual-use (Atlas, 2009), current debates are concerned primarily with cases where the consequences of malevolent use would be especially severe (Selgelid, 2009).

The dual-use dilemma is not new. When physicists observed atomic fission and the nuclear chain reaction early in the twentieth century, they realized that these discoveries might have beneficial applications in medicine and energy production; but they also realized that they could lead to the production of new, horribly efficient weapons. The manufacture and use of the first atomic bombs—and the nuclear arms race that followed—demonstrated that their fears were justified. According to the American biologist Matthew Meselson, this is not specific to nuclear physics: "Every major technology—metallurgy, explosives, internal combustion, aviation, electronics, nuclear energy—has been intensively exploited, not only for peaceful purposes but also for hostile ones" (Meselson, 2000). Similarly, recent advances in biology and genetics in particular raise the possibility of a new generation of biological weapons.

One of the most cited examples of dual-use research is that of Australian researchers who inadvertently developed a lethal mouse virus. In this now-famous study, the researchers used standard genetic engineering techniques to insert the gene for interleukin-4 (IL-4) into the mousepox virus. They hoped that the altered virus would induce infertility in mice—which are a major pest in Australia—and would thus serve as an infectious contraceptive for pest control. To their surprise, they discovered that the altered virus could kill both mice that were naturally resistant to, and mice that had been vaccinated against ordinary mousepox. When they published their findings, along with a description of the materials and methods, in the *Journal of Virology* in 2001 (Jackson *et al*, 2001), critics complained that they had thereby alerted would-be terrorists to new ways of making biological weapons and had provided them with explicit instructions.

> **…recent advances in biology and genetics in particular raise the possibility of a new generation of biological weapons**

Of particular concern was the possibility that the same techniques used to engineer the mousepox virus could be applied to create more virulent forms of poxviruses that afflict humans, including a vaccine-resistant strain of smallpox; one of the most devastating diseases in human history. Although it was eradicated in the 1980s, fears remain that former Soviet stockpiles—or genetically reconstituted forms of the virus—could be put to use by nefarious agents.

Given the historical importance of the mousepox experiment, we conducted separate interviews with the two primary researchers involved in the project—Ronald Jackson and Ian Ramshaw—in order to gain their perspective on this research. Although the interviews were conducted separately on 13 and 14 February 2008, we asked many of the same questions to both scientists. For the reader's ease, we therefore present their answers to some questions side-by-side below.

Michael J. Selgelid & Lorna Weir: How did you originally become involved with mousepox research?

Ronald Jackson: I started working with CSIRO [Commonwealth Scientific and Industrial Research Organization] in 1988 on a project to enhance myxomatosis to control rabbit populations—the myxoma virus is the rabbit equivalent of mousepox. Because rabbits aren't as well studied as mice, a lot of the reagents that we needed weren't available for the rabbit but had just become available for the mouse.

Selgelid & Weir: Can you explain the significance of the rabbit and mice problems in Australia?

Jackson: Rabbits were introduced in the mid-1800s and very quickly became an agricultural pest. CSIRO successfully introduced myxoma virus into Australia in 1950, which reduced the rabbit population, but then the virus and the rabbits started this co-evolution of rabbits developing resistance to the disease and the virus attenuating very quickly in response. By the late 1980s, the virus was controlling rabbit populations only moderately—and it wasn't effective at controlling population outbreaks. In agricultural areas, rabbits can be controlled moderately effectively using poisons and warren destruction, which can keep the numbers down. However, in the more arid zones of Australia, where there are very few people and little or no intensive agriculture, rabbits can cause major ecological problems.

18 EMBO *reports* VOL 11 | NO 1 | 2010

©2010 EUROPEAN MOLECULAR BIOLOGY ORGANIZATION

（Selgelid MJ. EMBO Rep. 11:18–24, 2010）

- 著者2人への個別インタビュー -

- 多くの研究者が抱えている問題の一つは、（デュアルユース性の）脅威が何であるかわからないということです。
- デュアルユース問題を内包する実験はたくさんありますが、それを探している人でなければ、それを認識することはできません。
- ほとんどの人は、デュアルユース問題が目の前にあっても解らないでしょう。

日本学術会議の提言

提言

病原体研究に関するデュアルユース問題

平成２６年（２０１４年）１月２３日
日 本 学 術 会 議
基礎医学委員会
病原体研究に関するデュアルユース問題分科会

各研究機関による教育と管理

各研究機関にあっては、病原体研究の危険性を認知し、研究を実施するための教育を徹底する。**研究者養成の段階で科学・技術の用途の両義性に関する教育を行なう**ほか、すでに研究開発に携わっている**研究者・技術者に対しても本問題に関する教育の機会を提供する**。また、研究機関としても起こり得る危険性の限局化の方策を整備し、管理する。

学協会の役割

学協会にあっては、**研究者・技術者が本問題に適切に対処できるよう教育機会を設け**、広報活動を推進するとともに、論文審査体制のあり方等についても議論を深める。

［入手元］https://www.scj.go.jp/ja/info/kohyo/pdf/kohyo-22-t184-2.pdf

DURCに関する教育動画の構成

1. 日本学術会議の提言
2. 「デュアルユース」とは
3. 感染症研究のデュアルユース問題
4. 生物兵器禁止条約
5. オーストラリア・グループ
6. 感染症法による病原体所持規制
7. 家伝法による病原体所持規制
8. 国連安保理事会決議第1540号
9. バイオテロ事件［2例］
10. 米国のバイオセキュリティ強化
11. CIA報告書：より暗い生物兵器の未来
12. エクトロメリアウイルスの強毒化
13. ポリオウイルスの化学合成
14. フィンク・レポート
15. DURと懸念されるDUR（DURC）
16. 米国におけるDURCの監視
17. 検討事例：野兎病菌
18. DURCの機関内監視プロセス概要
19. H5N1 influenza virusの研究［2報］
20. 保健福祉省の公表した7項目基準
21. 機能獲得研究（GOFR）
22. オランダ：Dual-Use Quickscan
23. DURの論文例［5報］
24. アメリカ微生物学会のDURC審査
25. DUR論文出版時の論評
26. DUR論文審査時の出版社の限界

研究立案時のデュアルユース性の検討

研究責任者による研究計画の立案　⇐ 動物実験、遺伝子組換え

研究責任者が別紙質問表に沿ってデュアルユース性を確認
［質問項目にYes/Noで回答］

（部長等がバイオリスク管理委員長に相談）
（Yesかどうか判断できない場合も同様）

1項目以上Yes

全てNo

今のところ対応不要
（研究責任者は継続的に評価）

（WGの構成は研究内容に応じて委員長が決定）

（WGには研究責任者と委員長を含む）

ワーキンググループ（WG）で議論

Dual Use Researchに該当せずと判断

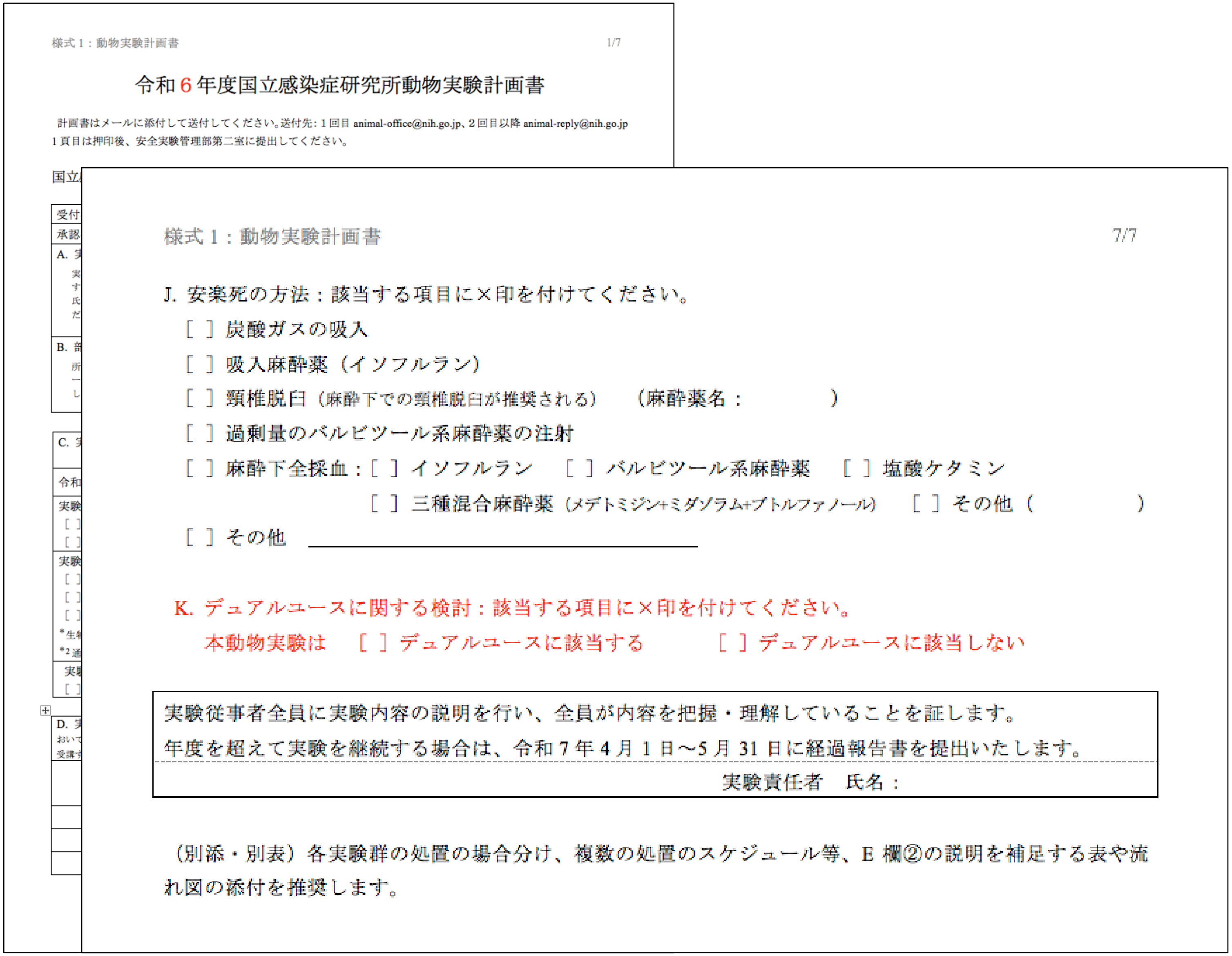
Dual Use Research（DUR）に該当と判断

研究責任者によるリスクマネジメント計画の策定

WGで審議、管理方針決定（必要に応じてWGに専門家を加える）

所長へ報告

計画段階でのデュアルユース性の検討確認



様式1：動物実験計画書 1/7

令和6年度国立感染症研究所動物実験計画書

計画書はメールに添付して送付してください。送付先：1回目 animal-office@nih.go.jp、2回目以降 animal-reply@nih.go.jp
1頁目は押印後、安全実験管理部第二室に提出してください。

国立

受付
承認
A. 実
B. 部
C. 実
令和
実験
実験
D. 実

様式1：動物実験計画書 7/7

J. 安楽死の方法：該当する項目に×印を付けてください。

[ ] 炭酸ガスの吸入
[ ] 吸入麻酔薬（イソフルラン）
[ ] 頸椎脱臼（麻酔下での頸椎脱臼が推奨される） （麻酔薬名： ）
[ ] 過剰量のバルビツール系麻酔薬の注射
[ ] 麻酔下全採血：[ ] イソフルラン [ ] バルビツール系麻酔薬 [ ] 塩酸ケタミン
[ ] 三種混合麻酔薬（メデトミジン+ミダゾラム+ブトルファノール） [ ] その他（ ）
[ ] その他 ______________________

K. デュアルユースに関する検討：該当する項目に×印を付けてください。

本動物実験は [ ] デュアルユースに該当する [ ] デュアルユースに該当しない

実験従事者全員に実験内容の説明を行い、全員が内容を把握・理解していることを証します。 年度を超えて実験を継続する場合は、令和7年4月1日～5月31日に経過報告書を提出いたします。
実験責任者 氏名：

（別添・別表）各実験群の処置の場合分け、複数の処置のスケジュール等、E欄②の説明を補足する表や流れ図の添付を推奨します。

答え

感染症（・病原体）研究者にデュアルユース問題を意識させるにはどうしたらよいか？

- デュアルユース問題についての学習機会を提供する。
- 研究申請様式へチェック欄を設けて意識させる。