図 3

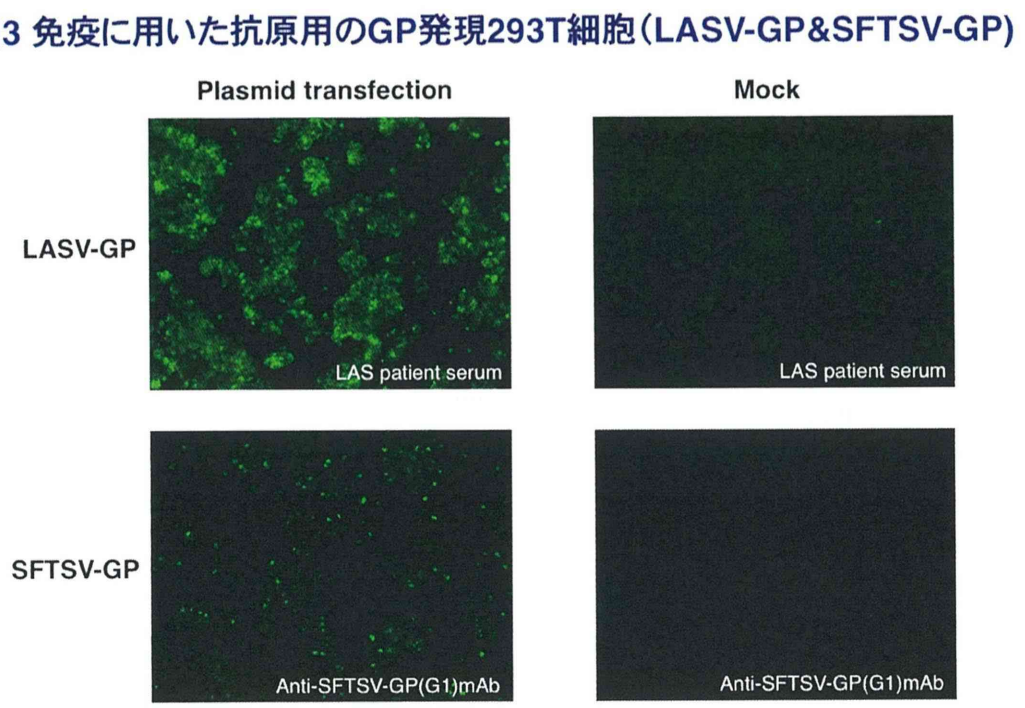


図3 免疫に用いた抗原用のGP発現293T細胞(LASV-GP&SFTSV-GP)
Plasmid transfection
Mock
LASV-GP
SFTSV-GP
LAS patient serum
LAS patient serum
Anti-SFTSV-GP(G1)mAb
Anti-SFTSV-GP(G1)mAb

図 4

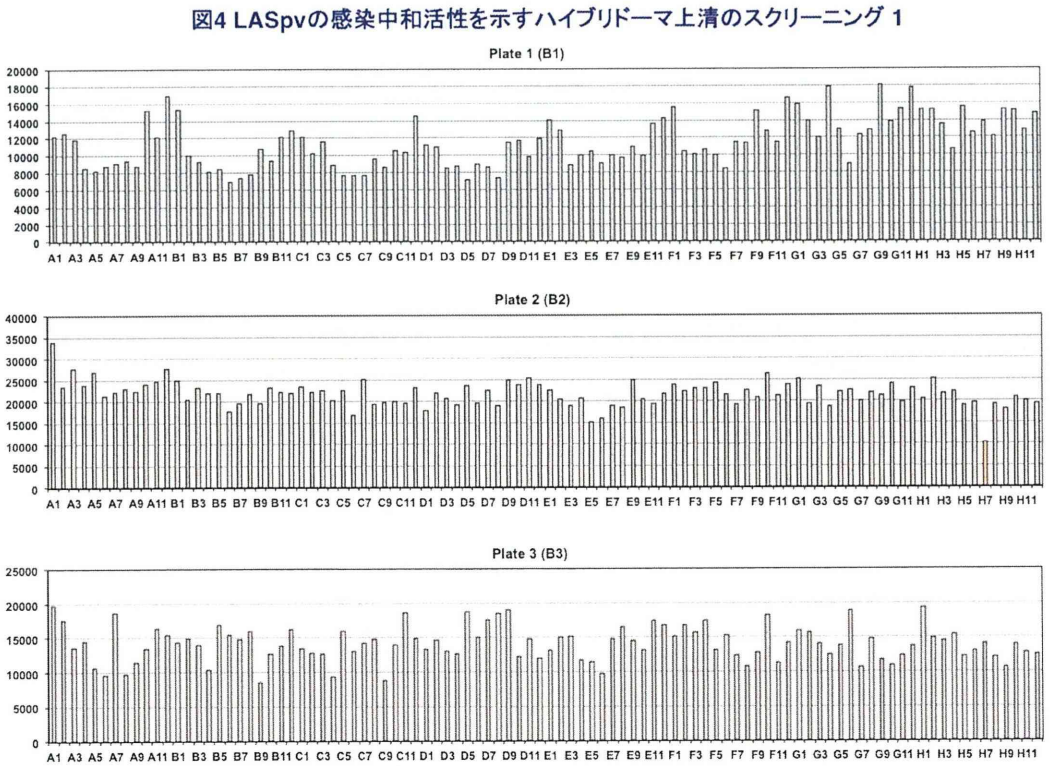


図4 LASpvの感染中和活性を示すハイブリドーマ上清のスクリーニング 1
Plate 1 (B1)
Plate 2 (B2)
Plate 3 (B3)
A1 A3 A5 A7 A9 A11 B1 B3 B5 B7 B9 B11 C1 C3 C5 C7 C9 C11 D1 D3 D5 D7 D9 D11 E1 E3 E5 E7 E9 E11 F1 F3 F5 F7 F9 F11 G1 G3 G5 G7 G9 G11 H1 H3 H5 H7 H9 H11

図4 LASpvの感染中和活性を示すハイブリドーマ上清のスクリーニング 2

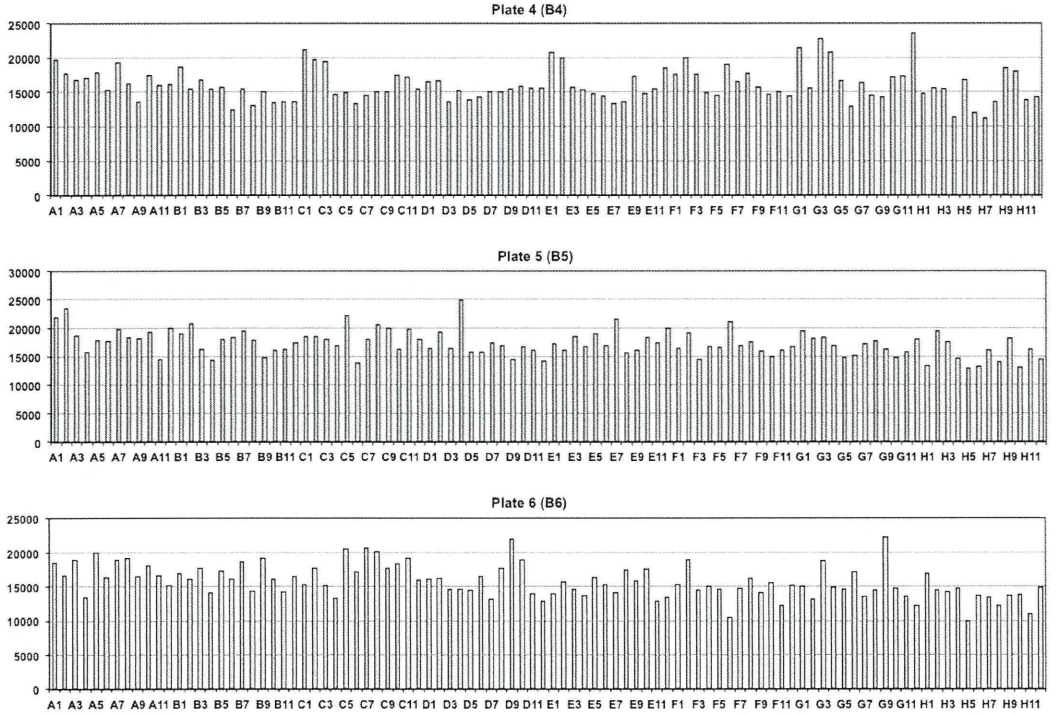



図4 LASpvの感染中和活性を示すハイブリドーマ上清のスクリーニング 3

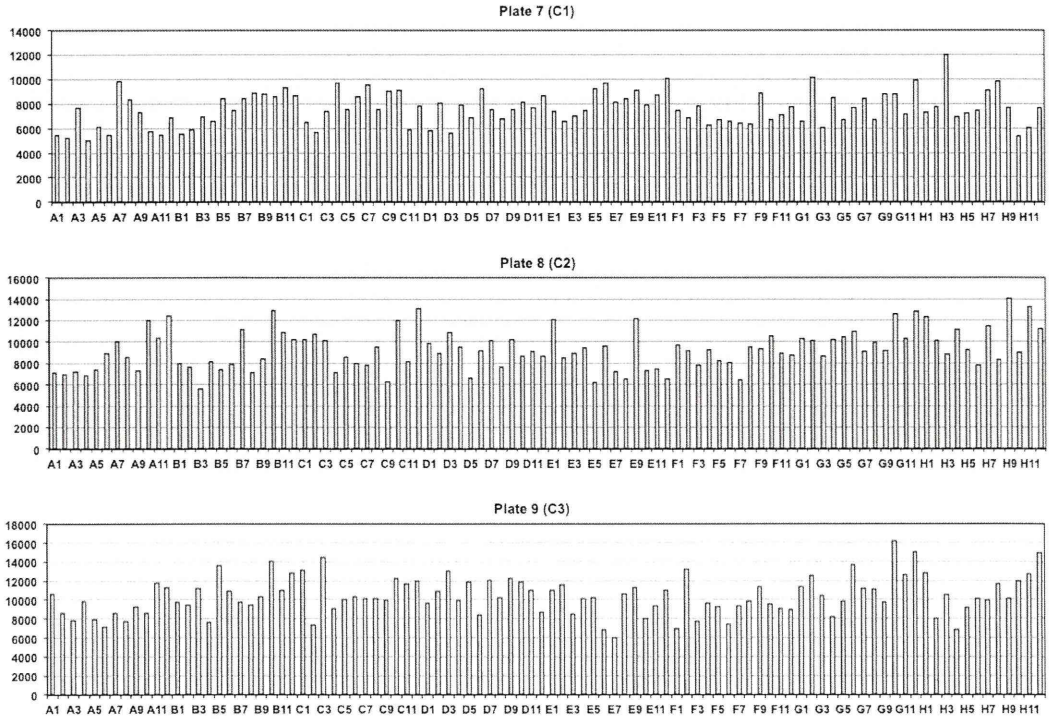


図4 LASpvの感染中和活性を示すハイブリドーマ上清のスクリーニング 4

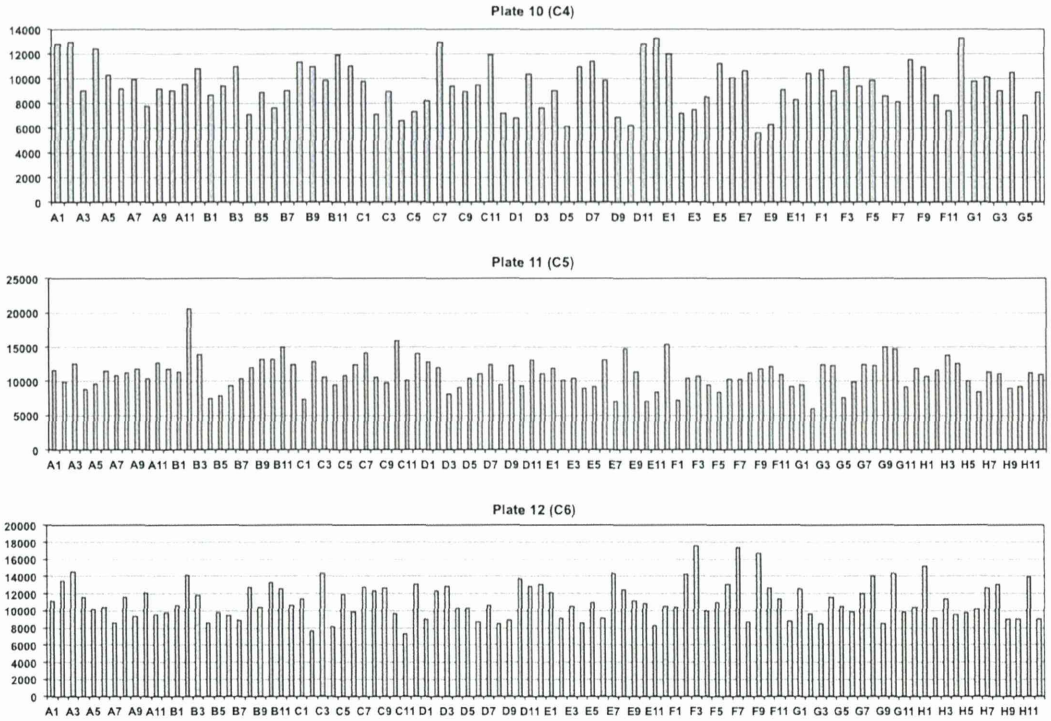



図 5

図5 SFTSVpvの感染中和活性を示すハイブリドーマ上清のスクリーニング 1

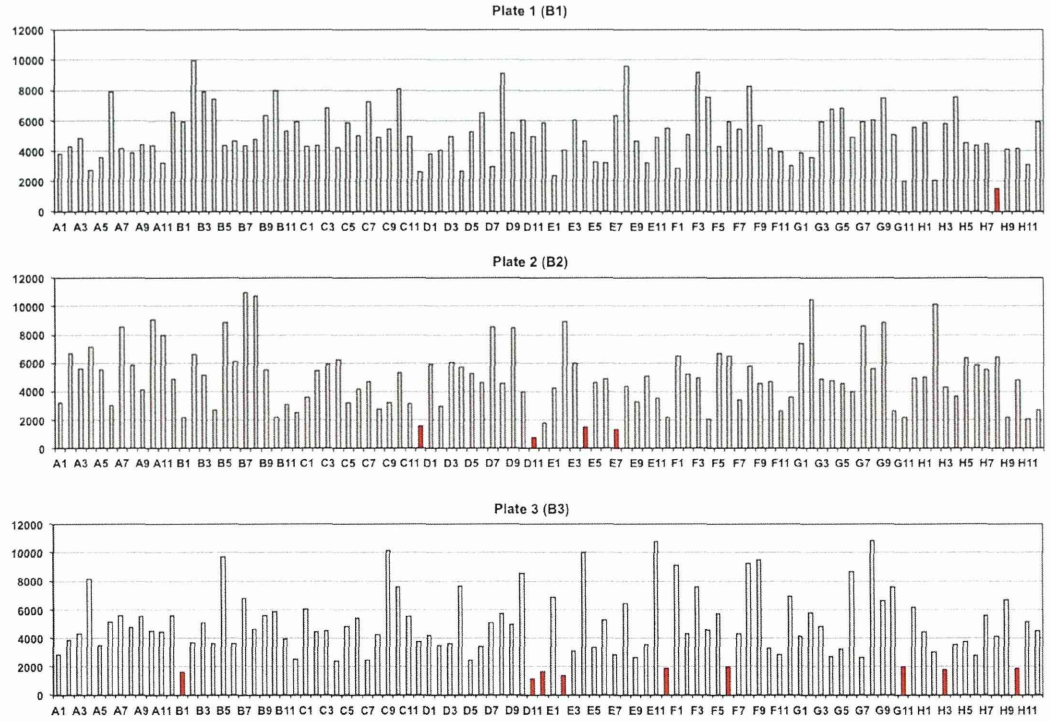


図5 SFTSVpvの感染中和活性を示すハイブリドーマ上清のスクリーニング 2

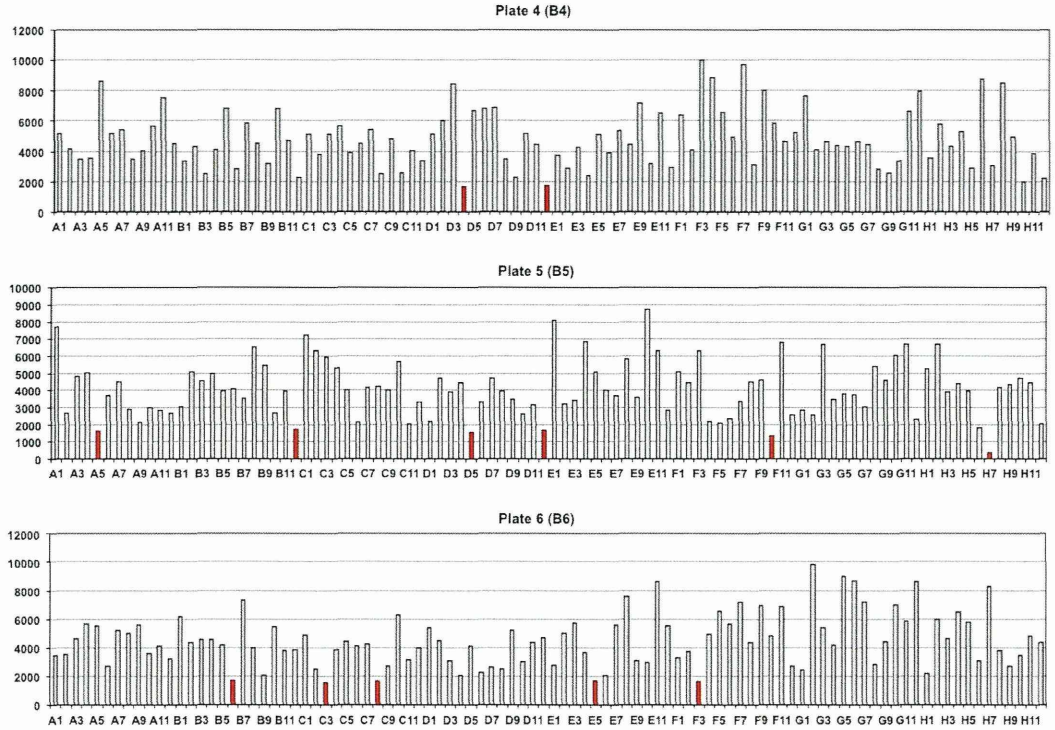



図5 SFTSVpvの感染中和活性を示すハイブリドーマ上清のスクリーニング 3

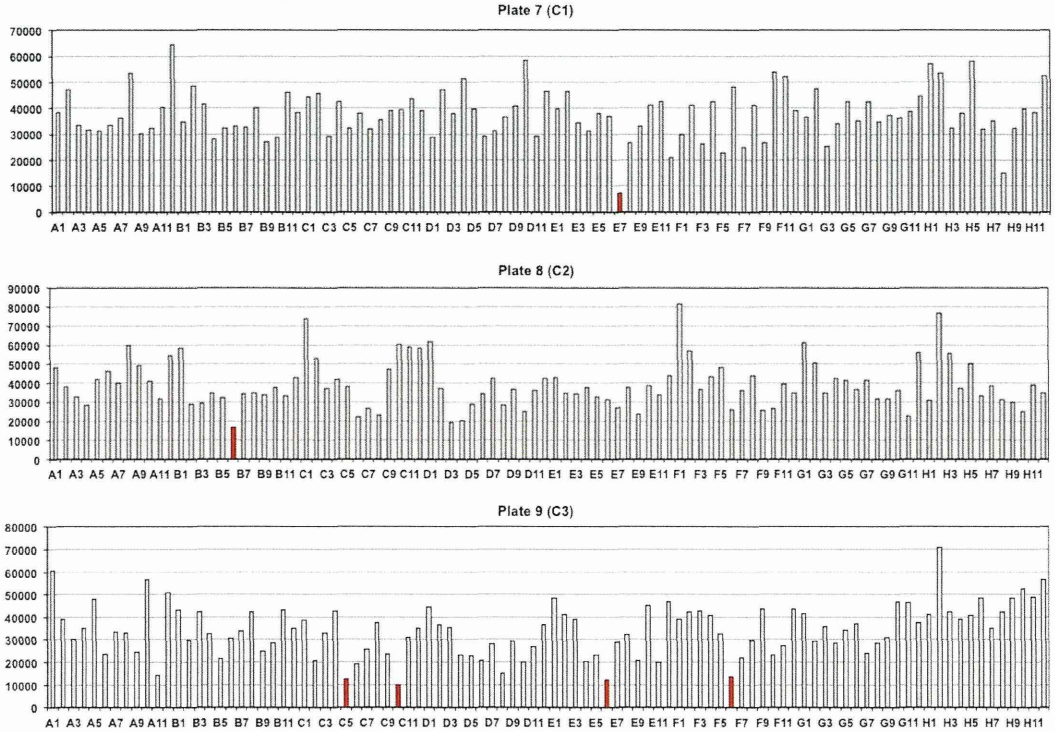


図5 SFTSVpvの感染中和活性を示すハイブリドーマ上清のスクリーニング 4

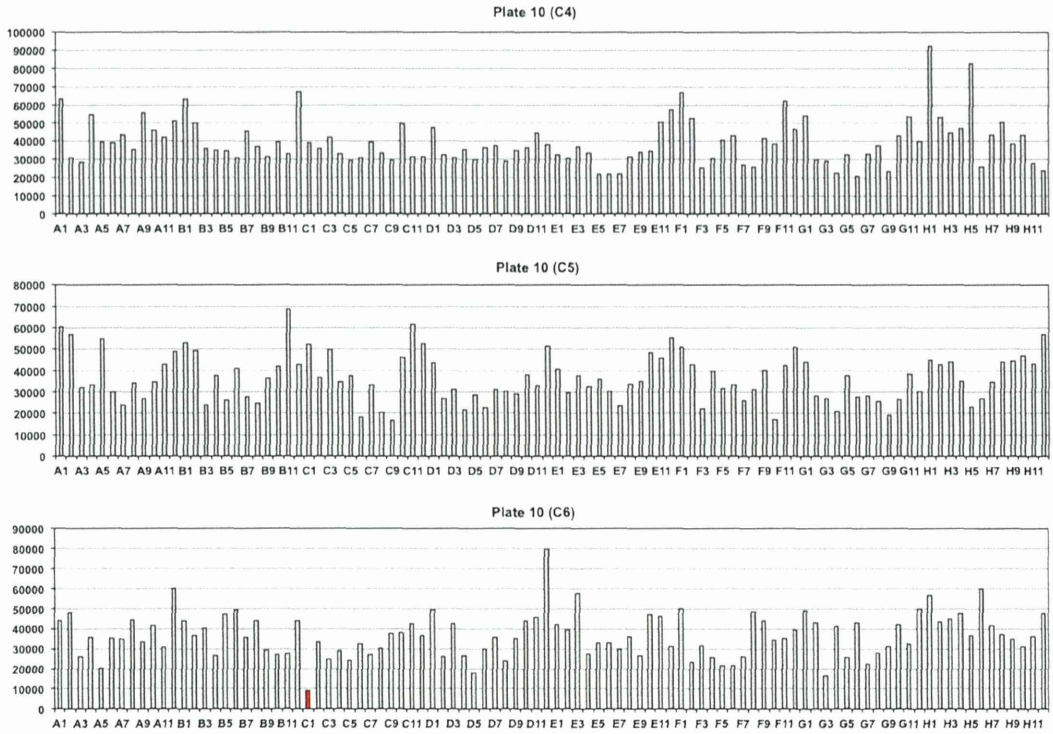



図 6

図6 SFTSVpvの感染中和活性を示すハイブリドーマ上清の選別
（2次スクリーニング）

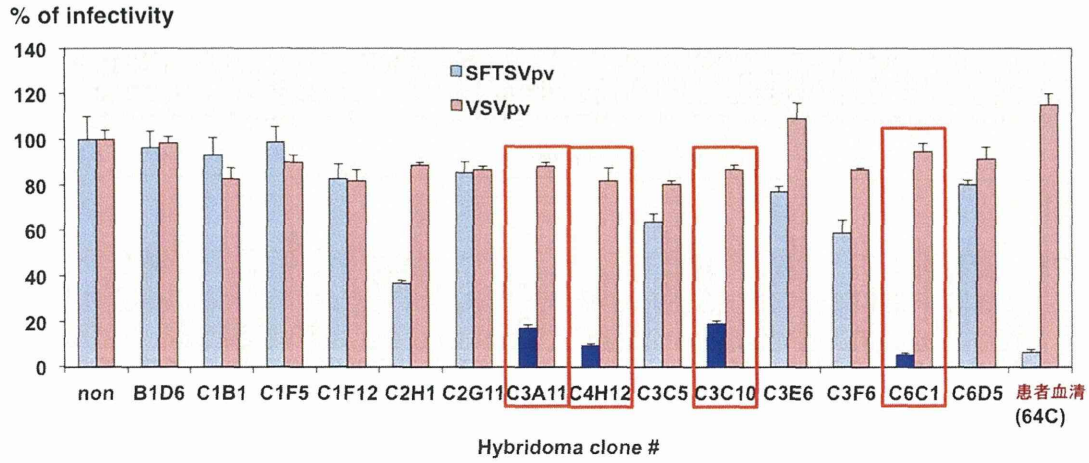


図 7

図7 SFTSV-GP発現HeLa細胞を用いた間接蛍光抗体法によるハイブリドーマ上清の選別

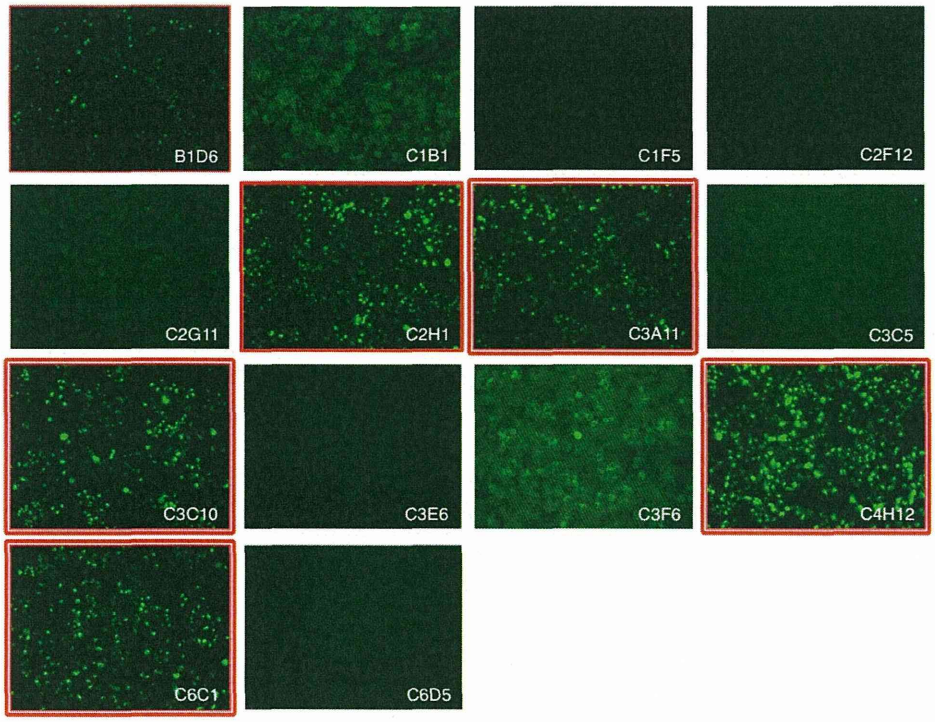



図7 SFTSV-GP発現NIH3T3細胞を用いた間接蛍光抗体法によるハイブリドーマ上清の選別

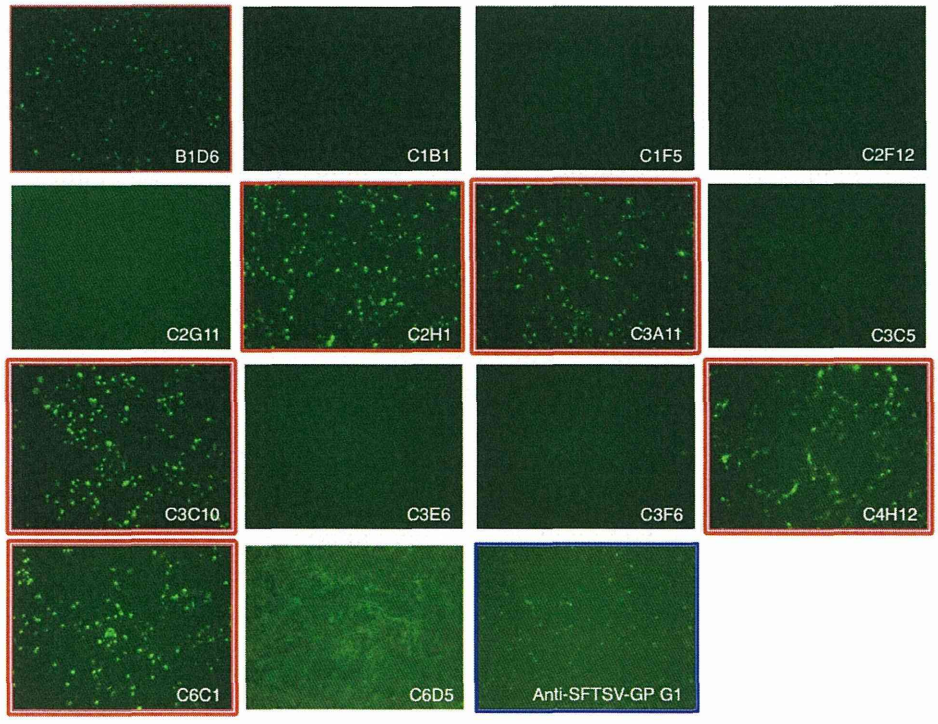


図7 LASV-GP発現HeLa細胞を用いた間接蛍光抗体法によるハイブリドーマ上清の選別

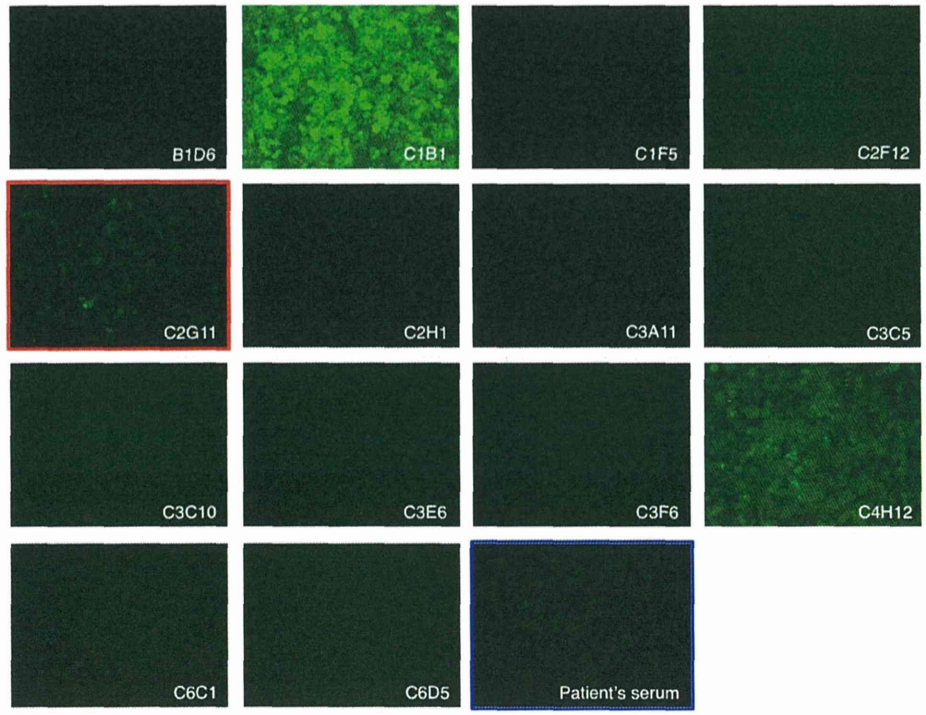



図7 LASV-GP発現NIH3T3細胞を用いた間接蛍光抗体法によるハイブリドーマ上清の選別

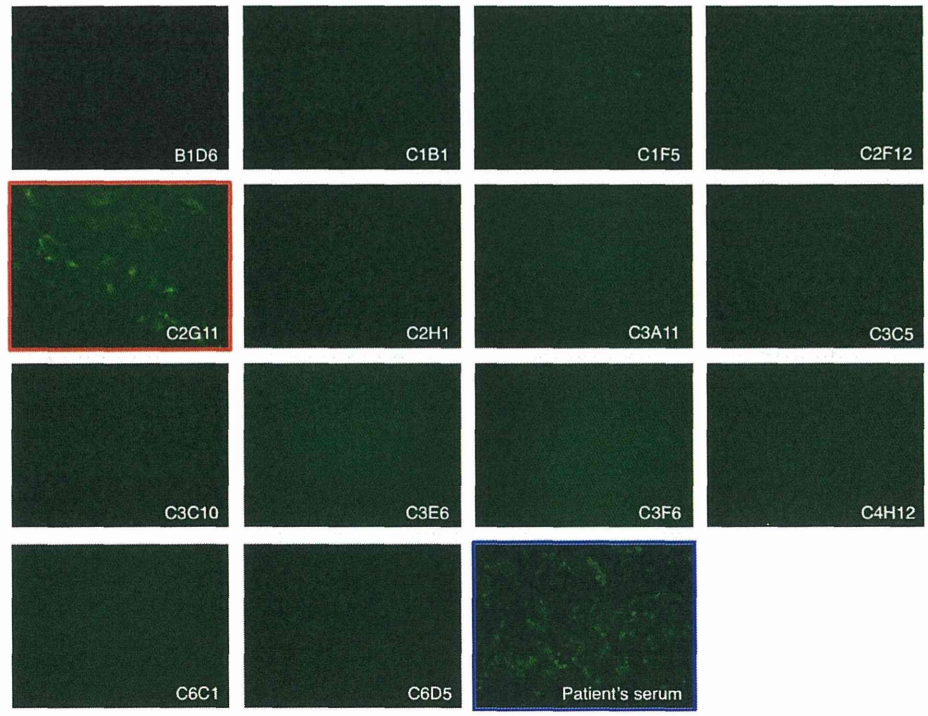


図 8

## 図8 SFTSV感染細胞を用いたELISA法によるハイブリドーマ上清の選別

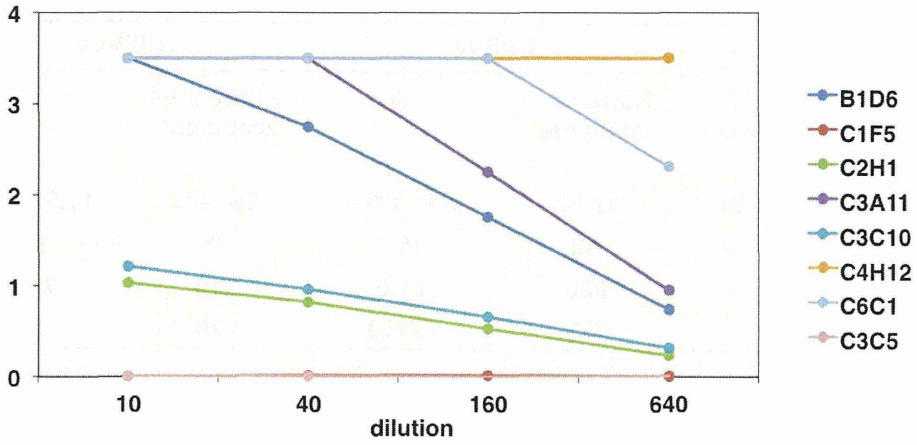
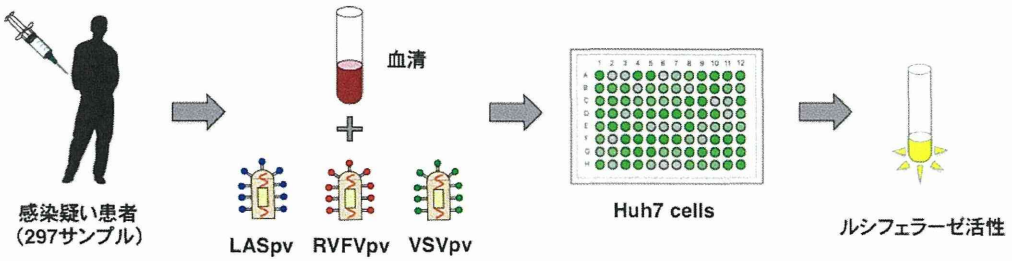



図 9

## シュードタイプウイルスを用いた患者血清中の抗体価測定



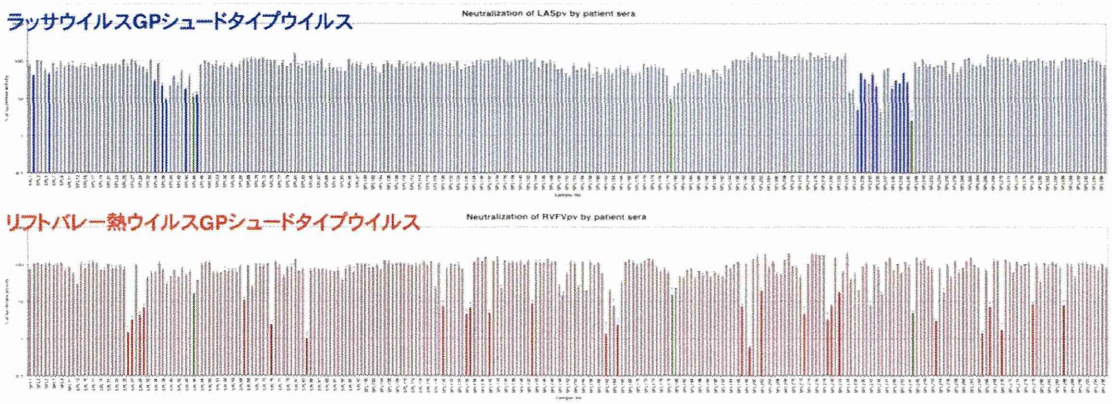



図 10

## ナイジェリア ボルノ州における 感染疑い患者血清中の抗体価測定

| % neutralization | Score | LASpv Number of samples* | LASpv % | RVFVpv Number of samples* | RVFVpv % |
|---|---|---|---|---|---|
| >80 (>90) | ++ | 11 (2) | 3.9 (18%) | 38 (24) | 13.6 (63%) |
| 50-80 | + | 42 | 15.1 | 25 | 9.0 |
| <50 | - | 226 | 81.0 | 216 | 77.4 |
| Total | | 279 | 100.0 | 279 | 100.0 |

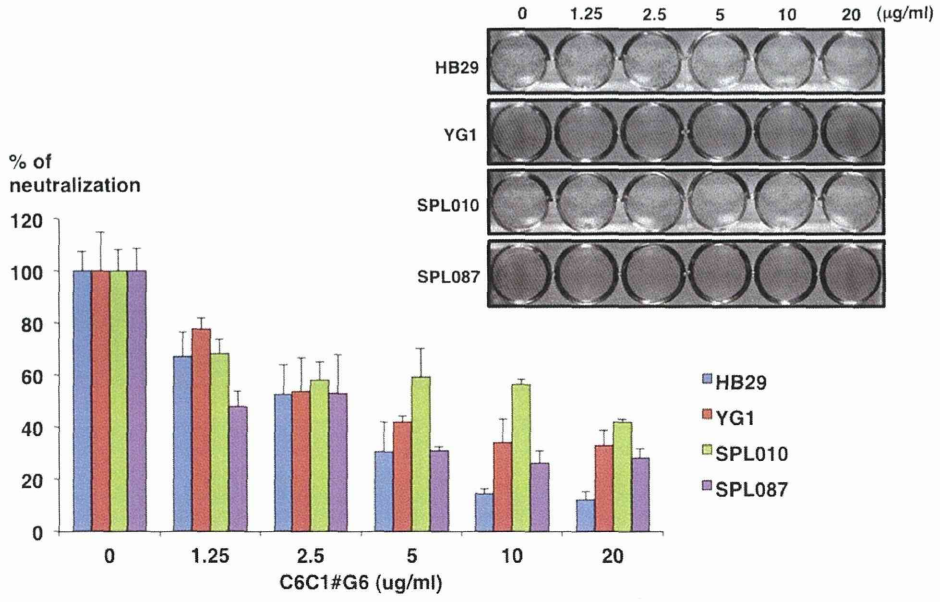
*18 samples showed evidence of microbial contamination, not included

LASpvはRVFVpvに比べて高い感染中和活性を示す血清が少ない

= ヒトでもラッサウイルスの感染では中和抗体が出来にくい!

図 11

## C6C1モノクローナル抗体によるSFTSVの感染中和




今後、中和エピトープの決定と動物実験での効果を検証予定

図 12

## C6C1モノクローナル抗体によるSFTSVpvの感染中和

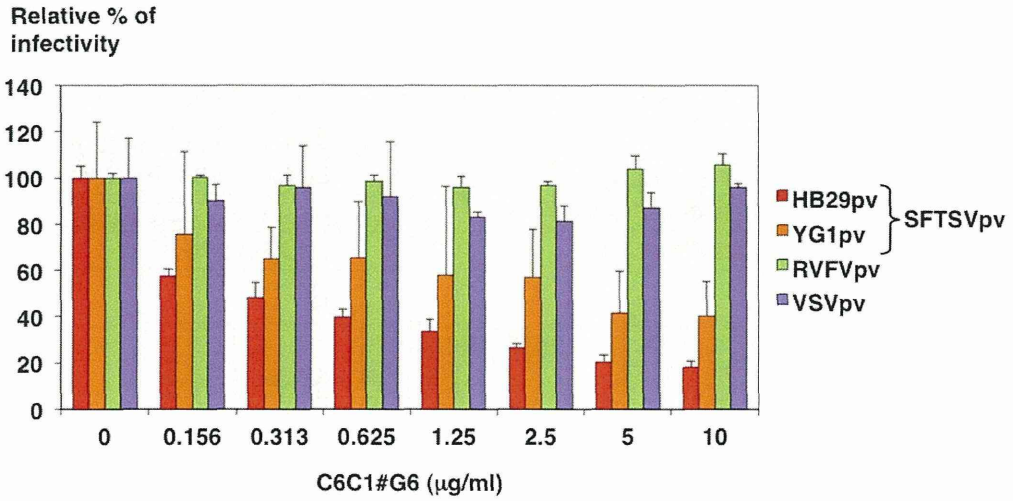
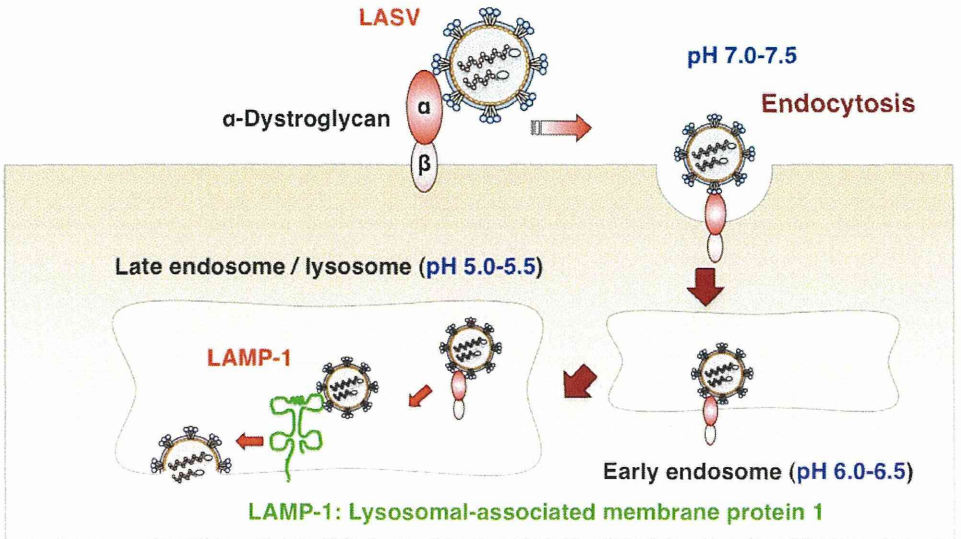



図 13

## ラッサウイルスの新規受容体

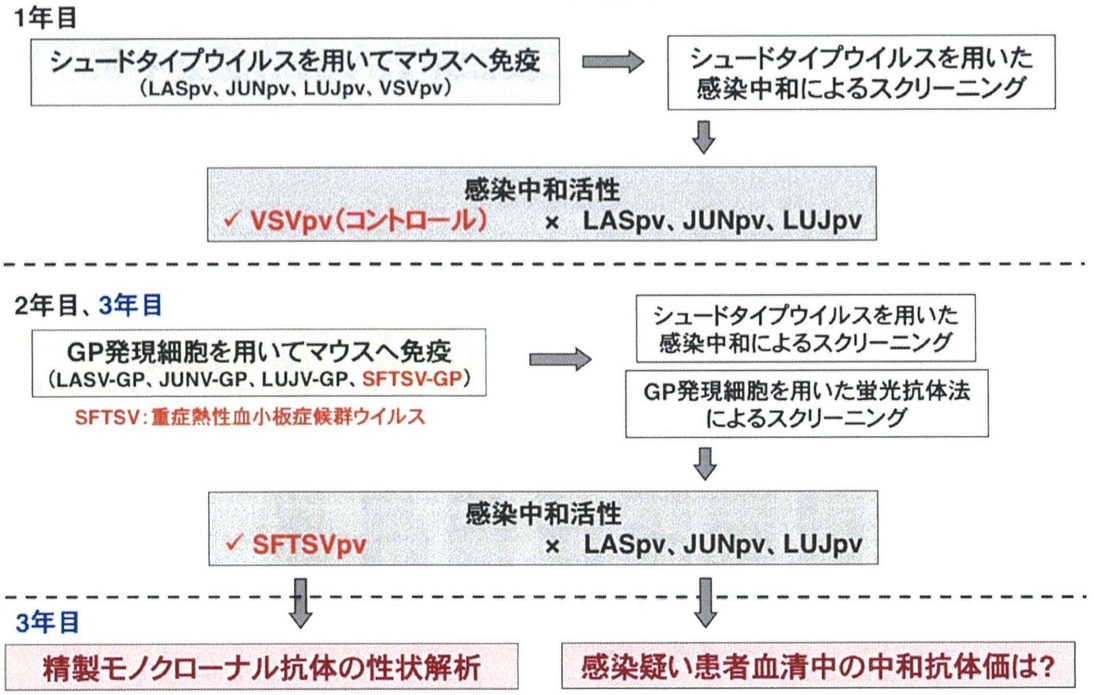
Jae et al., Science 2014




エンドソーム内でGPがLAMP-1に結合することが必要 = 感染が成立するには複雑な構造変化が必要

今後、GPの構造を変化させたものや受容体と結合させた状態での免疫を試してみる

# 研究成果概要


1年目
シュードタイプウイルスを用いてマウスへ免疫
（LASpv、JUNpv、LUJpv、VSVpv）
シュードタイプウイルスを用いた
感染中和によるスクリーニング
感染中和活性
✓ VSVpv（コントロール）　× LASpv、JUNpv、LUJpv
2年目、3年目
GP発現細胞を用いてマウスへ免疫
（LASV-GP、JUNV-GP、LUJV-GP、SFTSV-GP）
SFTSV：重症熱性血小板症候群ウイルス
シュードタイプウイルスを用いた
感染中和によるスクリーニング
GP発現細胞を用いた蛍光抗体法
によるスクリーニング
感染中和活性
✓ SFTSVpv　× LASpv、JUNpv、LUJpv
3年目
精製モノクローナル抗体の性状解析
感染疑い患者血清中の中和抗体価は？

# II. 研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

雑誌

| 発表者氏名 | 論文タイトル名 | 発表誌名 | 巻号 | ページ | 出版年 |
|---|---|---|---|---|---|
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| | | | | | |
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# III. 研究成果の刊行物・別刷

*Viruses* **2012**, *4*, 2097-2114; doi:10.3390/v4102097

OPEN ACCESS

*viruses*

**ISSN 1999-4915**

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*Review*

# Serological Assays Based on Recombinant Viral Proteins for the Diagnosis of Arenavirus Hemorrhagic Fevers

**Shuetsu Fukushi *, Hideki Tani, Tomoki Yoshikawa, Masayuki Saijo and Shigeru Morikawa**

Department of Virology, National Institute of Infectious Diseases, 4-7-1 Gakuen, Musashimurayama, Tokyo 208-0011, Japan; E-Mails: htani@nih.go.jp (H.T.); ytomoki@nih.go.jp (T.Y.); msaijo@nih.go.jp (M.S.); morikawa@nih.go.jp (S.M.)

* Author to whom correspondence should be addressed; E-Mail: fukushi@nih.go.jp; Tel.: +81-42-561-0771; Fax: +81-42-561-2039.

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**Abstract:** The family *Arenaviridae*, genus Arenavirus, consists of two phylogenetically independent groups: Old World (OW) and New World (NW) complexes. The Lassa and Lujo viruses in the OW complex and the Guanarito, Junin, Machupo, Sabia, and Chapare viruses in the NW complex cause viral hemorrhagic fever (VHF) in humans, leading to serious public health concerns. These viruses are also considered potential bioterrorism agents. Therefore, it is of great importance to detect these pathogens rapidly and specifically in order to minimize the risk and scale of arenavirus outbreaks. However, these arenaviruses are classified as BSL-4 pathogens, thus making it difficult to develop diagnostic techniques for these virus infections in institutes without BSL-4 facilities. To overcome these difficulties, antibody detection systems in the form of an enzyme-linked immunosorbent assay (ELISA) and an indirect immunofluorescence assay were developed using recombinant nucleoproteins (rNPs) derived from these viruses. Furthermore, several antigen-detection assays were developed. For example, novel monoclonal antibodies (mAbs) to the rNPs of Lassa and Junin viruses were generated. Sandwich antigen-capture (Ag-capture) ELISAs using these mAbs as capture antibodies were developed and confirmed to be sensitive and specific for detecting the respective arenavirus NPs. These rNP-based assays were proposed to be useful not only for an etiological diagnosis of VHFs, but also for seroepidemiological studies on VHFs. We recently developed arenavirus neutralization assays using vesicular stomatitis virus (VSV)-based pseudotypes bearing arenavirus recombinant glycoproteins. The goal of this article is to review the recent advances in developing laboratory diagnostic assays based on recombinant viral

proteins for the diagnosis of VHFs and epidemiological studies on the VHFs caused by arenaviruses.

**Keywords:** arenavirus; viral hemorrhagic fever; diagnosis; recombinant protein

## 1. Introduction

The virus family *Arenaviridae* consists of only one genus, but most viruses within this genus can be divided into two different groups: the Old World arenaviruses and the New World arenaviruses (also known as the Tacaribe complex) [1,2]. The differences between the two groups have been established through the use of serological assays. Most of the arenaviruses cause persistent infection in rodents without any symptoms, and humans acquire a variety of diseases when zoonotically infected. Lymphocytic choriomeningitis virus (LCMV) is the only arenavirus to exhibit a worldwide distribution, and causes illnesses such as meningitis [3,4]. Congenital LCMV infections have also been reported [4,5]. Most importantly, viral hemorrhagic fever (VHF) can be caused by several arenaviruses. Lassa fever, caused by the Lassa virus (LASV), an Old World arenavirus, is one of the most devastating VHFs in humans [6]. Hemorrhaging and organ failure occur in a subset of patients infected with this virus, and it is associated with high mortality. Many cases of Lassa fever occur in Western Africa in countries such as Guinea, Sierra Leone, and Nigeria [7–13]. Tacaribe complex lineage B of the New World arenaviruses consists of the Junin virus (JUNV), Guanarito virus (GUNV), Sabia virus (SABV) and Machupo virus (MACV), the etiological agents of Argentine, Venezuelan, Brazilian, and Bolivian hemorrhagic fevers, respectively [14,15]. Although genetically distinct from one another, they appear to produce similar symptoms, accompanied by hemorrhaging in humans [14,15]. These pathogenic New World arenavirus species are closely associated with a specific rodent species [6].

Humans are usually infected with pathogenic arenaviruses through direct contact with tissue or blood, or after inhaling aerosolized particles from urine, feces, and saliva of infected rodents. After an incubation period of 1–3 weeks, infected individuals abruptly develop fever, retrosternal pain, sore throat, back pain, cough, abdominal pain, vomiting, diarrhea, conjunctivitis, facial swelling, proteinuria, and mucosal bleeding. Neurological problems have also been described, including hearing loss, tremors, and encephalitis. Because the symptoms of pathogenic arenavirus-related illness are varied and nonspecific, the clinical diagnosis is often difficult [14,16]. Human-to-human transmission may occur via mucosal or cutaneous contact, or through nosocomial contamination [14,16]. These viruses are also considered to be potential bioterrorism agents [2].

A number of arenavirus species have been recently discovered as a result of both rodent surveys and disease outbreaks [17–26]. A novel pathogenic New World arenavirus, Chapare virus (CHPV), has been isolated from a fatal case of VHF in Bolivia [20]. In addition, five cases of VHF have been reported in South Africa, and a novel arenavirus, named Lujo virus, was isolated from a patient [17]. The Lujo virus is most distantly related to the other Old World arenaviruses [17]. To date, there is no information concerning the vertebrate host for the Chapare and Lujo viruses.