There is some evidence of endemicity of the Lassa virus in neighboring countries [27,28]. However, as the magnitude of international trade and travel is continuously increasing, and the perturbation of the environment (due either to human activity or natural ecological changes) may result in behavioral changes of reservoir rodents, highly pathogenic arenaviruses could be introduced to virus-free countries from endemic areas. In fact, more than twenty cases of Lassa fever have been reported outside of the endemic region in areas such as the USA, Canada, Europe, and Japan [29–33]. It is of great importance to detect these pathogens rapidly and specifically in order to minimize the risk and scale of outbreaks of VHFs caused by arenaviruses. However, these arenaviruses are classified as biosafety level (BSL)-4 pathogens, making it difficult to develop diagnostic techniques for these virus infections in laboratories without BSL-4 facilities. To overcome these difficulties, we have established recombinant viral nucleoproteins (rNPs)-based serological assays, such as IgG-enzyme-linked immunosorbent assay (ELISA), indirect immunofluorescence assay (IFA), and antigen (Ag)-capture ELISA for the diagnosis of VHFs caused by highly pathogenic arenaviruses. Furthermore, virus neutralization assays using pseudotype virus-bearing arenavirus GPs have been developed. In this review, we describe the usefulness of such recombinant protein-based diagnostic assays for diagnosing VHFs caused by arenaviruses.

2. Currently Used Diagnostic Techniques for VHFs

In outbreaks of VHFs, infections are confirmed by various laboratory diagnostic methods. Virus detection is performed by virus isolation, reverse transcription-polymerase chain reaction (RT-PCR), and antigen-capture ELISA. It has been shown that monoclonal antibody panels against pathogenic arenaviruses are useful for detecting viral antigens on the virus-infected cells as well as for investigating of antigenic relationships of arenaviruses [34–36]. Detection of the virus genome is suitable for a rapid and sensitive diagnosis of VHF patients in the early stage of illness, and extensive reviews of such RT-PCR assays have been described [37,38]. More recently, progress in the RT-PCR method covering genetic variations of the hemorrhagic fever viruses (HFVs) [39,40] and a multiplexed oligonucleotide microarray for the differential diagnosis of VHFs have also been reported [41]. On the other hand, antibodies against these viruses can be detected by the indirect immunofluorescence assay (IFA), or IgG- and IgM-ELISA. An IFA detects the antibody in the serum, which is able to bind to the fixed monolayer of the virus-infected cells. Although the interpretation of immunofluorescence results requires experience, the assay has advantages over other methods, since each virus generates a characteristic fluorescence pattern that adds specificity to the assay compared to a simple ELISA readout. A serological diagnosis by the detection of specific IgM and IgG antibodies to the HFVs must be sensitive, specific and reliable, because a misdiagnosis can lead to panic in the general population. An IgM-specific ELISA is suitable for detecting recent infection, but the relevance of IgM testing for acute VHF depends on the virus and the duration of illness; specific IgM is not often present in the very early stage of illness, and patients who die of VHF often fail to seroconvert at all. An IgG-specific ELISA is efficacious, not only in the diagnosis of a large number of VHF cases, especially during convalescence, but also for epidemiological studies in the endemic regions. The detailed methods used for the IFA and IgG- and IgM-ELISAs for the diagnosis of VHF using authentic virus-antigens have been described in detail [42–45].

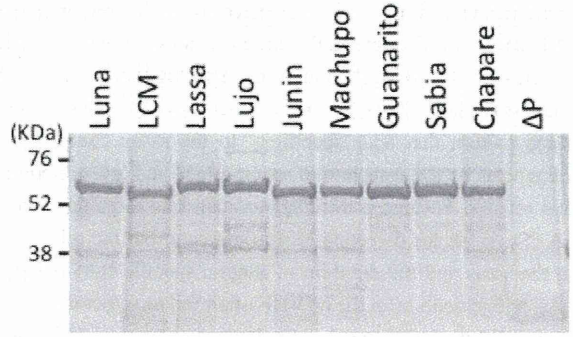
3. Recombinant Protein-Based ELISA for Detecting Antibodies against Arenaviruses

Arenaviruses have a bisegmented, negative-sense, single stranded RNA genome with a unique ambisense coding strategy that produces just four known proteins: a glycoprotein, a nucleoprotein (NP), a matrix protein (Z), and a polymerase (L) [46]. Of these proteins, the NP is the most abundant in virus-infected cells. Recombinant protein technology could meet the demand for a simple and reliable VHF test system, and recombinant NP (rNP) has been shown to be useful for serological surveys of IgM- and IgG antibodies against arenaviruses [47–50].

3.1. Antibody Detection-ELISA

Recombinant baculoviruses that express the full-length rNP of arenaviruses have been generated [48,50,51]. The method used for the purification of arenavirus rNP from insect *Tn5* cells infected with recombinant baculoviruses is effective and simple compared to those for Ebola, Marburg, and Crimean-Congo hemorrhagic fever virus rNPs [51–55]. Most of the arenavirus rNPs expressed in insect cells using the recombinant baculoviruses are crystallized [56] and are solubilized in PBS containing 8M urea. Since the majority of *Tn5* cellular proteins are solubilized in PBS containing 2M urea, the arenavirus rNPs in the insoluble fraction in PBS containing 2M urea can be solubilized by sonication in PBS containing 8M urea. After a simple centrifugation of the lysates in PBS containing 8M urea, the supernatant fractions can be used as purified rNP antigens without further purification steps [51]. The control antigen is produced from *Tn5* cells infected with baculovirus lacking the polyhedrin gene (ΔP) in the same manner as the arenavirus rNPs (Figure 1).

Figure 1. Purified rNPs. The expression and purification efficiency of arenavirus rNP were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) after staining the gels with Coomassie blue. Purified NP antigens with approximate molecular weights of 62 kDa from Luna, LCM, Lassa, Lujo, Junin, Machupo, Guanarito, Sabia, and Chapare viruses and the purified negative control antigen (ΔP) are shown.



As described above, recombinant baculoviruses allow the delivery of rNP antigens without using infectious live arenaviruses. An ELISA plate coated with the predetermined optimal quantity of purified rNPs (approximately 100 ng/well) is used for the IgG-antibody detection assay. An advantage

of using recombinant rNP for the IgG-ELISA is that it enables a direct comparison of antibody cross-reactivity among arenavirus rNPs, since antigen preparations of all arenavirus rNPs tested are performed using the same method [51]. Rabbit anti-sera raised against LCMV-rNP and LASV-rNP show cross-reactivity to LASV-rNP and LCMV-rNP, respectively, indicating that rabbit antibodies against rNPs of Old World arenaviruses cross-react with rNPs of other Old World arenaviruses (Table 1) [51]. Similarly, rabbit anti-sera generated against JUNV-NP show cross-reactivity to the LASV-rNP and LCMV-rNP, although the reaction is weak. However, rabbit anti-sera against LASV-NP and LCMV-NP show a negative reaction to the JUNV-rNP (Table 1) [51], indicating that rabbit antibodies against JUNV (a pathogenic New World arenavirus) NP might cross-react with the Old World arenavirus NP, whereas antibodies against Old World arenavirus NPs may not be able to react with pathogenic New World arenavirus NPs.

The rNP-based IgG-ELISA has also been used for the characterization of a mouse monoclonal antibody (MAb). Nakauchi *et al.* [50] have investigated the cross-reactivity of MAbs against JUNV rNP to pathogenic New World arenavirus rNPs, as well as LASV rNP. MAb C11-12 reacts at the same level with the rNPs of all of the pathogenic New World arenaviruses, including JUNV, GTOV, MACV, SABV, and CHPV, indicating that this MAb recognizes an epitope conserved among pathogenic New World arenaviruses. Another MAb, C6-9, reacts specifically with the rNP of JUNV, but does not react with those of the other pathogenic New World arenaviruses [50]. This indicates that MAb C6-9 recognizes a JUNV-specific epitope. None of these MAbs reacts with the rNP of the human pathogenic Old World arenavirus LASV. Thus, the MAb C11-12 is considered to be a broadly reactive MAb against New World arenaviruses, whereas MAb C6-9 is JUNV-specific. These findings have been confirmed by detailed epitope analyses using peptide mapping [50]. Similarly, the cross-reactivity of MAbs against LASV rNP has been analyzed [51]. MAb 4A5 cross-reacts with the Mopeia virus (MOPV) but not with the LCMV rNP. MAb 6C11 cross-reacts with LCMV rNP, while MAb 2-11 does not cross-react with LCMV rNP [51].

Table 1. Anti-serum reactivity for rNPs of different arenaviruses in IgG ELISAs.

Rabbit anti-serum	Reactivity for rNP from		
	LASV	LCMV	JUNV
anti-LASV NP	++	+	−
anti-LCMV NP	+	++	−
anti-JUNV NP	+	+	++

It is important to evaluate whether rNP-based ELISA is useful for the diagnosis of human VHF cases. The specificity of the LASV-rNP-based IgG ELISA has been confirmed by using sera obtained from Lassa fever patients [51]. The Lassa fever patients' sera show a highly positive reaction in the LASV-rNP-based IgG-ELISA, but sera from patients with Argentine hemorrhagic fever (AHF), which is caused by JUNV, do not. The serum from an AHF patient showed a highly positive reaction in the JUNV-rNP-based IgG-ELISA [49]. In addition, it was shown that, using sera obtained from AHF cases, the results of the JUNV rNP-based IgG ELISA correlate well with an authentic JUNV antigen-based IgG ELISA [49]. An IgM-capture ELISA using purified LASV-rNP as an antigen has been developed in the same way as in previous reports [54,57] and detects an LASV-IgM

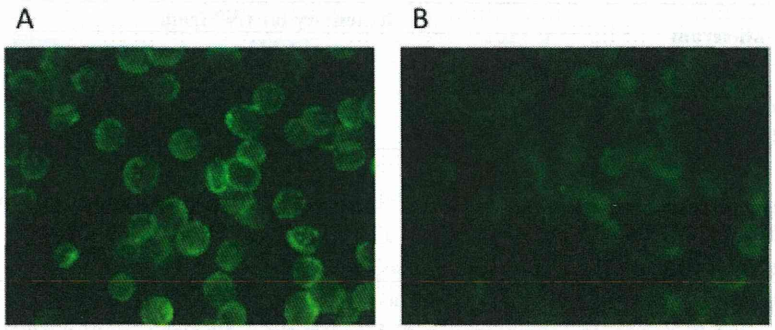
antibody [58]. In addition, immunoblot assays based on N-terminally truncated LASV rNP have been developed for detecting IgG and IgM antibodies against LASV. These methods may provide a rapid and simple Lassa fever test for use under field conditions [47].

3.2. Antibody Detection IFA

An IFA using virus-infected cells is a common antibody test for VHF viruses [59–63]. To avoid the use of highly pathogenic viruses for the antigen preparation, mammalian cells expressing recombinant rNP have been developed [51,57,64–68]. Lassa virus NP antigen for IFA can be prepared simply as described [51]. Briefly, the procedure involves (1) transfecting HeLa cells with a mammalian cell expression vector inserted with the cloned NP cDNA; (2) expanding the stable NP-expressing cells by antibiotic selection; (3) mixing the rNP-expressing cells with un-transfected HeLa cells (at a ratio of 1:1); (4) spotting the cell mixtures onto glass slides, then drying and fixing them in acetone.

In the IFA specific for LASV-NP, antibody positive sera show characteristic granular staining patterns in the cytoplasm (Figure 2) [69], thus making it easy to distinguish positive from negative samples. The specificity of the assay has also been confirmed by using sera obtained from Lassa fever patients [51]. In addition, an IFA using JUNV rNP-expressing HeLa cells has been developed to detect antibodies against JUNV, and the assay has been evaluated by using AHF patients' sera [70]. The LASV-rNP-based antibody detection systems such as ELISA and IFA are suggested to be useful not only for the diagnosis of Lassa fever, but also for seroepidemiological studies of LASV infection. In our preliminary study, approximately 15% of the sera collected from 334 Ghanaians and less than 3% of 280 Zambians showed positive reactions in the LASV-rNP-based IgG ELISA [58]. These results are in agreement with the fact that Lassa fever is endemic to the West African region, including Ghana, but less in the East African region.

Figure 2. Staining patterns of the LASV-rNP-expressing HeLa cells obtained from the sera of a Lassa-NP-immunized monkey (**A**) and control serum (**B**) in an IFA.



4. Antigen-Capture ELISA

For the diagnosis of many viral infections, PCR assays have been shown to have an excellent analytical sensitivity, but the established techniques are limited by their requirement for expensive equipment and technical expertise. Moreover, the high degree of genetic variability of the RNA viruses, including arenavirus and bunyavirus, poses difficulties in selecting primers for RT-PCR assays that can detect all strains of the virus. Since the sensitivity of the Ag-capture ELISA is comparable to that of RT-PCR for several virus-mediated infectious diseases, including Lassa fever and filovirus hemorrhagic fever [51,71–73], the Ag-capture ELISA is a sophisticated approach that can be used for the diagnosis of viral infections. Ag-capture ELISAs detecting viral NP in viremic sera have been widely applied to detect various viruses, since they are the most abundant viral antigens and have highly conserved amino acid sequences [50,51,54,71,72,74,75]. Polyclonal anti-sera or a mixture of MAbs present in the ascetic fluids from animals immunized for HFVs have been used for capture-antibodies in the Ag-capture ELISA [36,76–79]. MAbs recognizing conserved epitopes of the rNP are also used as capture antibodies since they have a high specificity for the antigens, and an identification of the epitopes of these MAbs is of crucial importance for the assessment of the specificity and cross-reactivity of the assay system [50,51,53,54,71,75]. In order to develop a sensitive diagnostic test for Lassa fever and AHF, rNPs of LASV and JUNV (see above) have been prepared, and newly established MAbs against them have been characterized and used for Ag-capture ELISAs [50,51]. The Ag-capture ELISA using MAb 4A5 has been confirmed to be useful in the detection of authentic LASV antigen in sera serially collected from hamsters infected with LASV [51]. The sensitivity of the MAb 4A5-based Ag-capture ELISA was similar to that of conventional RT-PCR, suggesting that the Ag-capture ELISA can be efficiently used in the diagnosis of Lassa fever [51]. Therefore, the MAb 4A5- based Ag-capture ELISA is considered to be useful in the diagnosis of Lassa fever. Also, by using MAbs raised against the rNP of JUNV, Ag-capture ELISAs specific for JUNV and broadly reactive to human pathogenic New World arenaviruses have been developed [50]. The Ag-capture ELISA using MAb E4-2 and C11-12 detected the Ags of all of the pathogenic New World arenaviruses tested, including JUNV. On the other hand, the Ag-capture ELISA using MAb C6-9 detects only the JUNV Ag. Considering that the symptoms of JUNV infection in humans are indistinguishable from those due to other pathogenic New World arenaviruses, the Ag capture ELISA using MAb C6-9 may be a useful diagnostic tool, especially for AHF [50].

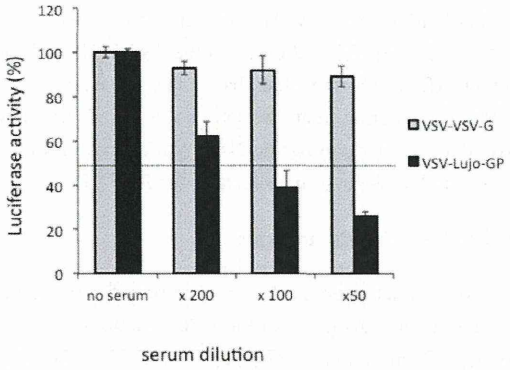
5. Neutralization Assays Based on VSV Pseudotypes

The virus neutralization assay is accepted as the "gold standard" serodiagnostic assay to quantify the antibody response to infection and vaccination of a wide variety of viruses associated with human diseases [80–86]. The presence of neutralizing antibodies is a reliable indicator of protective immunity against VHF [87–89]. The most direct method for detection of neutralizing antibodies against HFVs is by plaque reduction neutralization tests using infectious viruses. However, because of the high pathogenicity of HFVs to humans and the strict regulation of select agents, only a limited number of laboratories are able to perform such neutralization tests. For many HFVs, replication-incompetent pseudotyped virus particles bearing viral envelope protein (GP) have been shown to mimic the

respective HFV infections, thus, neutralization assays using the pseudotypes may be advantageous in some laboratory settings for the detection of antibodies to HFVs without the need for heightened biocontainment requirements.

The VSV-based vector has already been used to generate replication-competent recombinant VSVs to study of the role of GPs of various viruses [90–92]. Recent advances in producing pseudotype virus particles have enabled the investigation of the virus cell entry, viral tropism, and effect of entry inhibitors, as well as measurement of the neutralization titers, by using human immunodeficiency virus-, feline immunodeficiency virus-, murine leukemia virus-, or VSV-based vectors [86,93–103]. Pseudotypes based on VSV have advantages compared with other pseudotypes based on retroviruses for the following reasons. First, the pseudotype virus titer obtained with the VSV system is generally higher than that of the pseudotyped retrovirus system [104]. Second, the infection of target cells with a VSV pseudotype can be readily detected as green fluorescent protein (GFP)-positive cells at 7–16 h post-infection because of the high level of GFP expression in the VSV system [104,105]. In contrast, the time required for infection in the pseudotyped retrovirus system is 48 h [106,107], which is similar to the time required for infectious viruses to replicate to a level that results in plaque-forming or cytopathic effects in infected cells. A high-throughput assay for determining neutralizing antibody titers using VSV pseudotypes expressing secreted alkaline phosphatase [108,109] or luciferase (Figure 3) has also been developed.

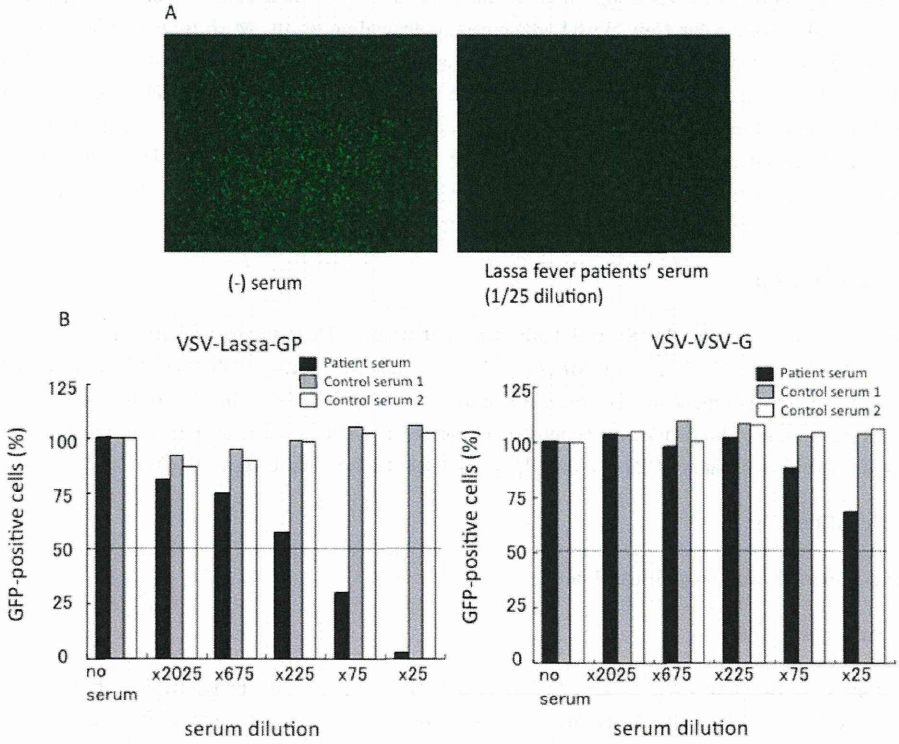
Figure 3. Neutralization assay for VSV-Lujo-GP. VSV-Lujo-GP or a control pseudotype (VSV-VSV-G) that expressed luciferase was incubated with serially diluted serum obtained from a rabbit immunized with Lujo-GPC, and was then inoculated in triplicate into Vero E6 cells. The luciferase activity (%) of each well compared to the negative control (no serum) is shown.



We have recently developed a VSV-based pseudotype bearing Lassa virus GP (VSV-LAS-GP) for the detection of neutralizing antibodies in the sera obtained from a Lassa fever patient. An example of the LASV neutralization assay using the VSV pseudotype is shown (Figure 4). In the presence of serum from Lassa fever patients, the number of GFP-positive cells (infectivity of VSV-LAS-GP) is significantly reduced compared with the number in the absence of the patient's serum (Figure 4A). The

control VSV pseudotype bearing VSV GP (VSV-VSV-G) is not neutralized by any sera. When the cut-off serum dilution is set at 50% inhibition of infectivity compared with the infectivity in the absence of the test serum, the neutralization titer of this patient's serum for VSV-LAS-GP is calculated to be 75 (Figure 4B). Likewise, a VSV-based pseudotype bearing the Junin virus GP has been developed for the detection of neutralizing antibodies from AHF patients' sera. The accuracy of the results of VSV-based neutralization assays has been confirmed by comparison with the results of the neutralization assay using live Junin virus [70].

Figure 4. Neutralization assay for VSV-Lassa-GP. (**A**) VSV-LAS-GP was incubated with or without serum obtained from a Lassa patient, and then was inoculated into Vero cells. The GFP signal was observed under a fluorescence microscope. (**B**) VSV-LAS-GP or the control pseudotype (VSV-VSV-G) incubated with serially diluted patient serum or healthy control sera were inoculated into Vero E6 cells. The relative number of GFP-positive cells (%) compared with negative control cells (no serum) is shown.



The Lujo virus is a new member of the hemorrhagic fever-associated arenavirus family from Zambia and southern Africa, and the virus is classified as a BSL-4 pathogen [17]. The genome sequence analysis of the Lujo virus suggests that the virus is genetically distinct from previously characterized arenaviruses. In order to study the infectivity of this newly identified arenavirus, we have

recently developed a luciferase-expressing VSV pseudotype bearing Lujo virus GPC (VSV-Lujo-GP). As shown in Figure 3, infection with VSV-Lujo-GPC is specifically neutralized by rabbit anti-Lujo GPC serum. Thus, the VSV-Lujo-GP may be a useful tool not only for determining the neutralizing antibody titer within the serum, but also for exploring yet-to-be-defined cellular receptor(s) for Lujo virus infection or for screening inhibitors of the Lujo virus GP-mediated cell entry.

6. Conclusions

Hemorrhagic fever outbreaks caused by pathogenic arenaviruses result in high fatality rates. A rapid and accurate diagnosis is a critical first step in any outbreak. Serologic diagnostic methods for VHFs most often employ an ELISA, IFA, and/or virus neutralization assay. Diagnostic methods using recombinant viral proteins have been developed and their utilities for diagnosing of VHF have been reviewed. IgG- and IgM- ELISAs and IFAs using rNPs as antigens are useful for the detection of antibodies induced in the patients' sera. These methods are also useful for seroepidemiological surveys for HFVs. Ag-capture ELISAs using MAbs to the arenavirus rNPs are specific for the virus species or can be broadly reactive for New World arenaviruses, depending on the MAb used. Furthermore, the VSV-based pseudotype system provides a safe and rapid tool for measuring virus neutralizing antibody titers, as well as a model to analyze the entry of the respective arenavirus in susceptible cells without using live arenaviruses. Recent discoveries of novel arenavirus species [17,26,110] and their potential to evolve predominantly via host switching, rather than with their hosts [110,111], suggest that an unknown pathogenic arenavirus may emerge in the future, and that the diagnostic methods for VHF caused by arenaviruses should thus be further developed and improved.

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Conflict of Interest

The authors declare no conflict of interest.

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特集　Negative Strand RNA Virusのウイルス学

6. アレナウイルス感染症

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アレナウイルスはアレナウイルス科に属するウイルスの総称で，細胞内で増殖し，ウイルス粒子内に宿主細胞のリボゾームが取り込まれ，これが砂状に見えるのでラテン語の砂粒(arenosus)にちなんで命名された．感染症法において1類感染症に指定されているラッサ熱を引き起こすラッサウイルス，南米出血熱の原因ウイルスとしてフニンウイルス，グアナリトウイルス，サビアウイルス，マチュポウイルス，チャパレウイルスなどが，ヒトに強病原性のアレナウイルスとして知られている．いずれも一種病原体に指定されている．また最近では，2008年にアフリカ南部地域で小規模なウイルス性出血熱が流行し，新規のアレナウイルス（ルジョウイルス）が同定された．日本では1987年のラッサ熱患者の1症例を除きアレナウイルスによる出血熱患者の発生はないが，他のウイルス性出血熱と同様に，いつ我が国で輸入症例が発生してもおかしくない状況であることから，病状や致死率を考えると診断や治療を行えるように整備しておく必要がある．本稿では，アレナウイルス感染症について，基礎研究から診断方法，ワクチン開発までを広く概説する．

はじめに

アレナウイルス科アレナウイルス属は，現在，アフリカ大陸を起源とする旧世界アレナウイルスと主に南アメリカ大陸を起源とする新世界アレナウイルスに分類され，約30種類のウイルスが同定されている（表1）[1)]．現在までそれ以外の地域での発生例は認められておらず，我が国においてもこれらのウイルスは存在しないため，なじみの薄いウイルス感染症のひとつである．しかしながら，比較的感染者の多いラッサウイルスは，ラッサ熱として西アフリカの流行地以外の地域での発生患者数が30人報告されており，ヨーロッパ各国や米国をはじめ世界中で輸入感染例が確認されている[2,3)]．日本でも1987年にシエラレオーネからの帰国者がラッサ熱を発症した事例が報告されている[4)]．

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出血熱の原因となるアレナウイルスは，他の出血熱ウイルスと同様にバイオセーフティレベル(BSL)4に分類され，BSL4実験施設での取り扱いが必要とされる．我が国でも国立感染症研究所に高度安全研究施設が設置されているものの，現在までBSL4としては稼働されていないために，感染性ウイルスを用いての実験ができない．BSL4実験施設を備えているような諸外国においても施設内での実験における取り扱いの煩雑さから，アレナウイルス感染症に対する基礎研究やワクチン開発などはなかなか進んでいない．実験室診断に関しては，我が国においては国立感染症研究所ウイルス第一部が，感染性ウイルスを用いない方法で多くのアレナウイルス感染症を診断するためのシステムを開発し備えている[5,6,7)]．

本稿では，アレナウイルス感染症についてこれまで明らかにされてきたことをウイルス学的な観点から概説したい．

アレナウイルスの種類と感染症

アレナウイルス科アレナウイルス属に分類される約30種類のウイルスのうち，9種類がヒトに出血熱等の疾患を引き起こす[8)]．主に，齧歯類が宿主として同定されており，ヒトは感染宿主の尿や体液に含まれるウイルスを経気道経路で吸入することにより感染する．それぞれのウイルスは，表1に示す独自の宿主動物を有している．アレナウイルス

表1 アレナウイルスの分類と特徴（文献12を補足・改変）

	ウイルス(略記)	宿主動物	受容体候補（空欄は未同定）	分布地域	ヒトへの病原性（空欄は無しor不明）	BSL分類（特定病原体分類）
旧世界アレナウイルス	Dandenong	不明		オーストラリア		
	Ippy (IPPYV)	*Arvicanthus spp.*		中央アフリカ		
	Kodoko	*Nannomys minutoides*		ギニア		
	Lassa (LASV)(ラッサ)	*Mastomys sp.*	α-DG, LSECtin, DC-SIGN, Axl, Tyro3	西アフリカ	ラッサ熱	BSL4(一種)
	Lymphocytic choriomeningitis (LCMV)	*Mus domesticus, Mus musculus*		世界中	リンパ球性脈絡髄膜炎	BSL2
	Lujo (LUJV)(ルジョ or ルヨ)	不明	non α-DG, non-TfR1?	南アフリカ	出血熱	未定
	Mobala (MOBV)(モバラ)	*Praomys sp.*	α-DG	中央アフリカ		BSL2
	Mopeia (MOPV)(モペイア)	*Mastomys natalensis*	α-DG	モザンビーク,ジンバブエ		BSL2
	Morogoro	*Mastomys sp.*		タンザニア		
新世界アレナウイルス クレードA	Allpahuayo (ALLV)	*Oecomys bicolor, Oe.*		ペルー		
	Bear Canyon (BCNV)	*Peromyscus californicus*		アメリカ合衆国		
	Catarina	*Neotoma micropus*		アメリカ合衆国		
	Flexal (FLEV)	*Oryzomys spp.*		ブラジル	あり	
	Parana (PARV)	*Oryzomys buccinatus*		パラグアイ		
	Pichinde (PICV)(ピチンデ)	*Orzomys albigularis*		コロンビア		BSL2
	Pirital (PIRV)	*Sigmodon alstoni*		ベネズエラ		
	Skinner Tank	*Neotoma mexicana*		アメリカ合衆国		
	Whitewater arroyo (WWAV)	*Neotoma albigula*		アメリカ合衆国	出血熱	
クレードB	Amapari (AMPV)(アマパリ)	*Oryzomys capito, Neacomys guianae*	Non-TfR1	ブラジル		
	Chapare (CHPV)(チャパレ)	不明	TfR1	ボリビア	出血熱	BSL4(一種)
	Cupixi (CPXV)	*Oryzomys sp.*		ブラジル		(2011年1月印定)
	Guanarito (GTOV)(グアナリト)	*Zygodontomys brevicauda*	TfR1	ベネズエラ	ベネズエラ出血熱	BSL4(一種)
	Junin (JUNV)(フニン)	*Calomys musculinus*	TfR1	アルゼンチン	アルゼンチン出血熱	BSL4(一種)
	Machupo (MACV)(マチュポ)	*Calomys callosus*	TfR1	ボリビア	ボリビア出血熱	BSL4(一種)
	Sabia (SABV)(サビア)	不明	TfR1	ブラジル	ブラジル出血熱	BSL4(一種)
	Tacaribe (TCRV)(タカリベ)	*Artibeus spp.*	Non-TfR1	トリニダード	あり	
	Tamiami (TAMV)	*Sigmodon hispidus*		アメリカ合衆国		
クレードC	Latino (LATV)	*Calomys callosus*	α-DG	ボリビア		
	Oliveros (OLVV)	*Bolomys obscurus*	α-DG	アルゼンチン		
	Pampa (PAMV)	不明		アルゼンチン		
	Pinhal	*Calomys tener*		ブラジル		

*太字で記したウイルスはヒトに病原性を示し、アレナウイルス科において注目すべきウイルス。空欄はなし、もしくは不明、未定。

は抗原性などから大きく旧世界と新世界アレナウイルスに分類され，新世界アレナウイルスはさらにA, B, Cの3クレードに分類される（表1）．アレナウイルス感染症で最も重要なのはラッサウイルス感染によるラッサ熱で，毎年，中央～西アフリカにかけて流行し，数万人が感染，5,000人程度が死亡していると考えられている．ラッサ熱は，ウイルス性出血熱の中で輸入症例が最も多く，ラッサ熱流行地からの海外渡航者または帰国者が潜伏期間中に移動して流行地以外で発症する事例がたびたび報告されている[2,3]．同様に，新世界アレナウイルスに分類されるフニンウイルス感染によるアルゼンチン出血熱はかつて年間患者数が数百から3,500名ほど（致死率30%）いたが，1991年の生ワクチンの導入により1992年以降，患者発生数は年間30-50人と劇的に減少した[8]．その他の南米出血熱であるボリビア出血熱(マチュポウイルス)，ベネズエラ出血熱(グアナリトウイルス）およびブラジル出血熱（サビアウイルス）は症例こそ少ないものの，致死率は高い[9]．近年，新興アレナウイルスとしては，2003-2004年にボリビアのチャパレ川流域で発生した出血熱アウトブレイク時に患者から分離されたチャパレウイルス[10]と，2008年にザンビア共和国のルサカ(Lusaka)で発生し，南アフリカ共和国のヨハネスブルグ(Johannesburg)の病院で院内感染患者から分離されたLujo（ルジョもしくはルヨ）（ルサカとヨハネスブルグの頭文字を併せて命名）ウイルス[11]が報告されている．それぞれ，致死率は高いものの一時的なアウトブレイクで終息している．

その他，旧世界アレナウイルスには，ヒトには無菌性髄膜炎などを引き起こすリンパ球性脈絡髄膜炎ウイルス(LCMV)が存在する．このウイルスは，他のアレナウイルス属ウイルスと異なり世界中に広く分布しており，出血熱症状は起こさず致死率は高くないものの，最近は感染者からの臓器移植を受けた患者が感染し全員死亡する事例が数回報告されている．LCMVは古くから免疫学の基礎研究のモデルウイルスとしても利用されており，BSL2病原体であること，旧世界アレナウイルスに分類されるラッサウイルス等に比較的近い遺伝子配列を持つことから，現在もラッサウイルス研究のモデルウイルスとして利用されているだけでなく，様々な研究に使用されている．

アレナウイルスのゲノム構造と構成蛋白

アレナウイルスは，2分節（S-RNAとL-RNA）の1本鎖の（−）鎖RNAをゲノムに有するエンベロープウイルスで，S-RNAにNPとGPC（GP1, GP2前駆体）蛋白をコードする(図1)．NPは（−）センスに，GPCは（+）センスにコードされるというアンビセンス様式をとる[9]．それぞれの蛋白のmRNAはゲノムの反対側から転写され，その間にある安定したヘアピン構造を持つ非翻訳遺伝子間領域(Non-coding intergenic region; IGR）で終了する（図1）[9]．

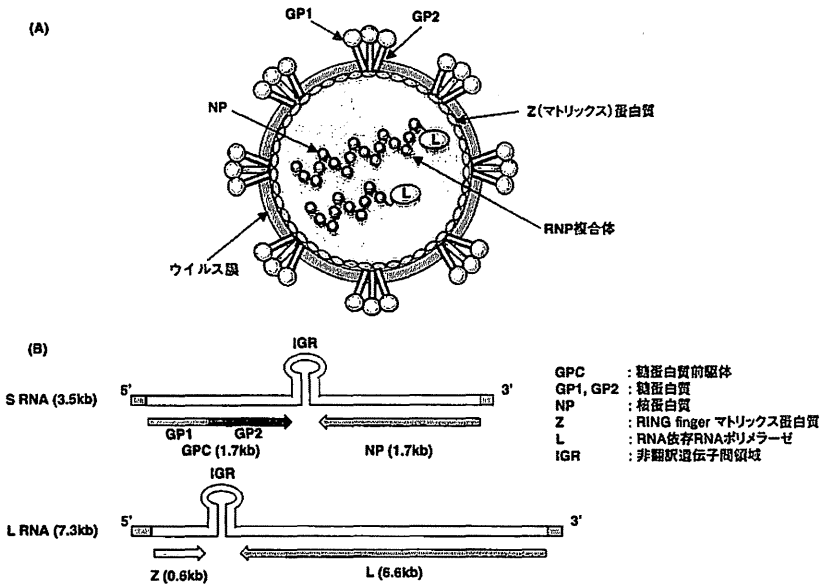

図1　アレナウイルスの粒子構造と遺伝子構成

(A) アレナウイルス粒子の模式図．通常ウイルスサイズは直径 50-300nm．ウイルス RNA はウイルス核蛋白質（NP）と共にリボ核蛋白質粒子（RNP）複合体を形成して粒子内に包含される．RNA 依存 RNA ポリメラーゼ（L）は，RNP と結合し，脱殻後，自身のゲノム転写時に作用する．Z 蛋白質はマトリックス蛋白としてウイルス膜内に結合し，GP2 の膜内領域部分と相互作用し，GP2 の成熟に関与している．(B) アレナウイルスのアンビセンスコード戦略（コーディングストラテジー）．2つの一本鎖 RNA 断片 S と L は，それぞれ中間部分に位置する非翻訳遺伝子間領域（IGR）によって2分割され，それぞれ反対方向から2種類の蛋白を合成するコーディングストラテジーをとる．

一方，L-RNA には，RNA 合成酵素である L 蛋白が（−）センスに，Z 蛋白が（+）センスに，それぞれコードされる[7]．NP は感染細胞内およびウイルス粒子内に最も多く存在し，ウイルス ribonucleoprotein (RNP) の主要な構成成分として，またウイルス RNA の転写・複製に必須な蛋白である．近年，アレナウイルス NP が1型インターフェロンの誘導を抑制することが明らかとなり[12]，ラッサウイルスの NP の結晶構造解析により NP の C 末領域がインターフェロン誘導抑制に関わる3'-5'エキソリボヌクレアーゼ活性を持ち，DEDDh ファミリーに似た構造を持っていることが示された[13,14]．GPC は，小胞体内でサブチラーゼ SKI-1/S1P により GP1 と GP2 に開裂され，ウイルススパイク上部に位置する GP1 が細胞膜表面に存在する受容体と結合し，GP1 と N 末側でイオン結合し，C 末側に膜貫通領域を持つ GP2 が受容体結合後の膜融合に関与すると考えられている[15]．Z 蛋白は，Zinc finger 蛋白で，他の非分節型 RNA ウイルスの M 蛋白に似た多機能な性状を持ち，RNA の転写や複製の阻害，蛋白質合成阻害，ウイルス粒子の成熟や出芽などに関与していることが証明されている[16,17]．これらの多機能性は Z 蛋白の構造的にフレキシブルな C 末端領域と種々の宿主因子（eIF4E, Tsg101 等）との結合によると考えられている[18]．

アレナウイルスの感染経路と自然宿主

アレナウイルスはいずれも齧歯類を自然宿主とし，ラッサウイルスはアフリカ大陸に広く分布するマストミス（*Mastomys natalensis*）を宿主とする（表1）[9]．ウイルスは持続感染したマストミスの排泄物や唾液に排出され，ヒトはマストミスによる咬傷，あるいは汚染物との直接接触により感染する．ヒトーヒト間の感染も血液，排泄物との直接接触により感染する．空気感染は証明されていない．マストミスはアフリカにおいては人と共棲的に生活し，住家性ネズミに近い状態で，家あるいはその周辺に生息し，人口の増加に伴いマストミス数も増加するものと考えら

れ，衛生状態の改善は必須な課題である．フニンウイルスもヨルマウスの一種である *Calomys musculinus* を自然宿主とし，同様の感染経路でヒトへ感染することが知られている．それぞれのウイルスと自然宿主においてどのように持続感染が成立するのか詳細は明らかにされていないが，いくつかのウイルスでは，同じ種類のネズミを自然宿主としており，これらの自然宿主への持続感染機構は似ているものと考えられる．

アレナウイルスの受容体

1990年代はじめに，LCMVの細胞受容体が120-150kDaの糖蛋白質であることが報告され[19]，続けてこの蛋白質が細胞表面に広く発現している細胞膜外表在性糖蛋白質であるαジストログリカン（α-DG）であることが明らかにされた[20]．α-DGは，膜貫通糖蛋白質であるβジストログリカンに結合することにより細胞膜に結合しており，細胞外マトリックスと細胞骨格を結ぶ連結軸として細胞膜の安定化に寄与していると考えられている．LCMVに続き，同じ旧世界アレナウイルスであるラッサウイルスやモバラウイルス，モペイアウイルスの他，新世界アレナウイルスのクレードCに属するウイルスもα-DGを初期受容体として利用していることが報告されている（表1）[21]．しかしながら，α-DGのリガンドであるラミニンがラッサウイルスのGPCとDGの結合を阻害するものの，ラッサウイルスの感染を阻害することはできず，他の受容体の存在も示唆されていた[22]．最近，α-DG以外の受容体としてC型レクチンファミリーのLSECtinとDC-SIGN，またTAM受容体チロシンキナーゼファミリーのAxlとTyro3が新たな受容体候補として報告された[23]．これらの受容体は，ラッサウイルスだけでなくLCMVの細胞侵入にも関与していることが明らかとなった[24]．AxlとTyro3はエボラウイルスの細胞侵入にも関与することが既に明らかとなっており，細胞指向性や病原性との関連も興味深い[25,26]．

新世界アレナウイルスの受容体もマチュポウイルスのGP1蛋白質を用いたプルダウン法によりトランスフェリン受容体1（TfR1）が同定された[27]．TfR1は，マチュポウイルスだけでなく，新世界アレナウイルスのクレードBに属するフニンウイルス，グアナリトウイルス，サビアウイルス，チャパレウイルスも受容体として利用していることが明らかとなった（表1）[27,28]．TfR1は，鉄を結合したトランスフェリンを細胞内に取り込むのに重要な受容体で，様々な細胞表面に発現しており，TfRには1と2が存在するが，TfR2はアレナウイルスの受容体としては機能しない[27]．また，現在までのところ，旧世界アレナウイルスではTfR1を受容体として利用する種は確認されておらず，新世界アレナウイルスと旧世界アレナウイルスでは，細胞侵入に関わる受容体分子も大きく違うことが明らかとなった．近年，新たに同定されたルジョウイルスに関しては，まだ受容体は同定されておらず，α-DGでもTfR1でもないことが示唆されている．新世界アレナウイルスのクレードBに属するウイルスの中にもTfR1非依存的な感染を示すウイルス（アマパリ，タカリベウイルス）が存在しており[29,30]，さらに，LCMVの変異体にはα-DG非依存的な感染を示す株もあり[31]，こうしたウイルスが利用している未知な新規受容体の同定も期待される．また，これまで受容体が明らかにされてきたウイルスでも，宿主特異性や病原性を決定付けるような第二，第三の受容体の存在も否定できず，今後の研究が期待される．

アレナウイルスの細胞侵入機構

アレナウイルスの生活環に関する基礎的研究は，感染性ウイルスを利用し難い状況から，レトロウイルスや水疱性口内炎ウイルス（VSV）を基盤としたシュードタイプウイルスを利用して，受容体の同定を含めた細胞侵入機構の解析や，一部リバースジェネティクスが確立しているウイルスではレプリコンを用いた複製機構の解析などが進められている．遺伝子構成や転写，複製機構に関しては他の総説を参照していただき[9,32]，本稿では主に細胞侵入機構について近年明らかになってきたことを紹介したい．

アレナウイルスは，前述の通り，α-DGやTfR1などの結合受容体に結合した後に，主にエンドサイトーシスによって細胞内に取り込まれ，低pH環境下で細胞の膜とウイルス膜の膜融合が起こり，脱殻され，ウイルスRNP複合体が細胞質内に放出される．LCMVやラッサウイルスは，クラスリン，カベオリン，ダイナミン，アクチンなど一般的なエンドサイトーシスに関わる分子に非依存的で，代わりに，SV40などのウイルスが利用するカベオラ／ラフト経路とは異なるコレステロール依存的な経路で侵入すると考えられている[21,33]．なお，α-DGへの結合にはコレステロールは必要としないため，結合以降の段階でコレステロールが関与していると思われる[34]．最近，これらのウイルスの感染に，腔内膜小胞（intraluminal vesicles; ILV）や多胞体（multivesicular body; MVB）の形成に関与するリゾビスフォスファチジン酸やフォスファチジルイノシトール3-キナーゼが重要であることが報告され，また，Alixなどの ESCRT 関連蛋白質も感染に必要であることから，初期エンドソームを介さず直接MVBや後期エンドソームを介した経路を利用して細胞内輸送されることが提唱された[35]．初期エンドソームを介さないことは，インターフェロン応答の誘導を回避する手段の一つとしても考えられ，α-DGを介した旧世界アレナウイルスの感染経路と免疫応答の関連性についても興味深い．

一方，TfR1は，トランスフェリンがクラスリン依存的なエンドサイトーシスを調べる際のマーカー分子として用いられるように，典型的なクラスリン依存的エンドサイトーシスの形態をとり，これらを受容体として利用する新