Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Virology

Animal models for Lassa virus infection

Tsing-Lee Tang-Huau[1], H Feldmann[1,2] and K Rosenke[1]

In humans, Lassa virus infection can result in disease with hemorrhagic manifestations and high fatality rates. There are no approved treatments or vaccines available and the inherent danger of studying Lassa virus means it can only be studied in high containment labs (BSL4). Under these conditions, mouse models are becoming an important instrument in the study of Lassa virus infection, disease and host responses. While guinea pigs and non-human primates are the critical components in assessing treatments and vaccines and have recently been used with great affect in this capacity.

Addresses
[1] Laboratory of Virology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rocky Mountain Laboratories, Hamilton, MT, USA
[2] Department of Medical Microbiology, University of Manitoba, Winnipeg, Manitoba, Canada

Corresponding authors: Feldmann, H (feldmannh@niaid.nih.gov), Rosenke, K (rosenkek@niaid.nih.gov)

Current Opinion in Virology 2019, **37**:112–117

This review comes from a themed issue on **Lassa viruses**

Edited by **Connie Schmaljohn** and **David Safronetz**

For a complete overview see the Issue and the Editorial

Available online 20th August 2019

https://doi.org/10.1016/j.coviro.2019.07.005

1879-6257/Published by Elsevier B.V.

Introduction

Lassa virus (LASV) was discovered and isolated in 1969 in the village of Lassa in Nigeria [1]. Shortly after identification of the virus, it was isolated from a small peridomestic rodent, *Mastomys natalensis*, which is currently known as the main natural reservoir of LASV [2]. LASV causes an acute febrile disease, called Lassa Fever (LF); in West Africa that in its severe form resembles the clinical syndrome of a viral hemorrhagic fever. The four West African countries Guinea, Sierra Leone, Liberia, and Nigeria have reported the vast majority of all LF cases, but neighboring countries do see varying numbers of LF cases and LASV seems to be epizootic in *Mastomys natalensis* and occasionally other rodent species [3,4]. LASV show a high genetic diversity with up to 27% nucleotide difference in the NP gene translating into a difference of up to 15% at the protein level [5]. There are now four recognized clades of LASV with two possible emerging clades that have not yet been formally recognized [5–8]. The newly developed LASV reverse genetics system will be a powerful tool in future studies on virus biology, pathogenesis and immune responses. [9,10]. Currently, there is no clinically approved treatment for Lassa Fever (LF) or a vaccine to prevent LASV infection. The only available antiviral drug, ribavirin, has been used with limited success if administered early in infection (within the first 6 days after disease onset) [11,12]. The outbreaks documented throughout West Africa have increased in size and severity over time [13–15]. This has also resulted in the importation of LF cases into Europe and the Americas resulting in the first transmission event of LASV outside of west Africa [14,15]. LF is a growing public health concern best demonstrated by adding LF/LASV to the WHO List of Blueprint Priority Diseases/Pathogens, which identifies those diseases/pathogens that have epidemic potential and a lack of vaccine or treatment options. This illustrates the continued need for basic and translational research that by necessity, occurs predominantly through the use of animal models.

Animal models

The guinea pig has historically been the main rodent model for studies on LASV pathogenesis and countermeasure development. The nonhuman primate (NHP) models have largely been used to further support and confirm results from the guinea pig model. The more recently developed mouse models are still in their infancies but show promise for more mechanistic studies on pathogenesis and immune response as well as screening models for therapies and vaccines. In addition, some early studies were performed in the reservoir species *Mastomys natalensis*. The different models are discussed in more details below (Table 1).

Mastomy natalensis

Shortly after the discovery of *Mastomys natalensis* as the main natural host of LASV it became one of the first animals experimentally infected and characterized [3]. These animals were found to support LASV replication over the course of several weeks following infection with no signs of disease [3] but there has been limited follow-up to these initial studies [16] and none under controlled experimental conditions utilizing an established *Mastomys natalensis* colony.

Guinea pigs

The most reliable small animal model of LF established thus far is the guinea pig. Both the inbred Strain 13 and outbred Hartley guinea pig are susceptible to wild-type (WT) Lassa infection by aerosol, sub cutaneous

Table 1

Comparison of Lassa Virus Josiah strain in infection models

		Mouse				Guinea pig			Non-human primate		
	Model	IFNAR$^{-/-}$	Humanized HHD	Chimeric	STAT1$^{-/-}$	Strain 13	Hartley		Rhesus macaque	Common marmoset	Cynomolgus macaque
Disease	Virus adaptation	No	No	No	No	No	No	Yes	No	No	No
	Viremia	Moderate[b]	High	High	High[a]	High	Moderate	High	Moderate	High[a]	High
	Target organ damage	Moderate	High	Moderate	High[a]	High	Moderate	High	High	High[a]	High
	Hearing loss	N/D	N/D	N/D	Yes	N/D	N/D	N/D	N/D	N/D	Yes
	Fever	N/D	N/D	N/D	N/D	Yes	Yes	Yes	Yes	Yes	Yes
Research	Cost	Moderate				Low			High		
	Handling	Easy				Moderate			Difficult		
	Group size	Large				Medium			Small		
	Tools and reagents	Commonly available				Minimally available			Commonly available		
	FDA anaimal rule requirement	N/D				Yes			Yes		

N/D: Not determine.
[a] Strain-dependent.
[b] Non-lethal.

or intraperitoneal routes ([3,17,18]). However, the Strain 13 is more sensitive to Lassa virus infection and results in a near 100% fatality rate when infected with the Josiah strain (Table 1). The Hartley guinea pig is more resistant with ~30% of animals succumbing to disease following infection with the Josiah strain [17]. More recently the Josiah strain was adapted to the Hartley guinea pig by serial passaging virus from lethally infected animals to naïve animals 4 times. This serial passaging resulted in a uniformly lethal infection in the Hartley guinea pig that is consistent in disease and with wild type virus in Strain 13 guinea pigs [19] (Table 1).

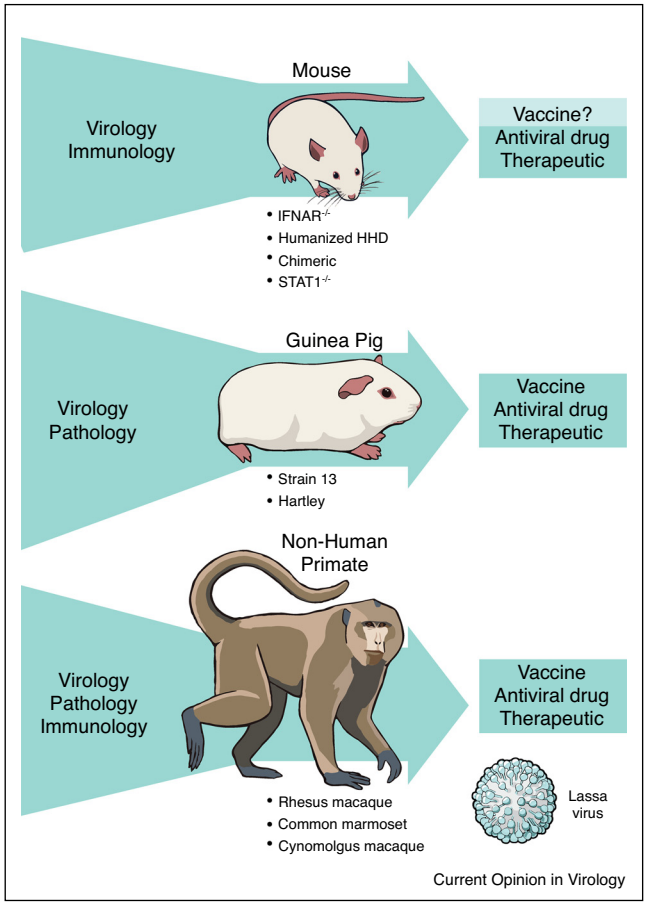
Viremia is detectable by qPCR as early as 3 days post-infection (dpi) in guinea pigs and peaks around 10–12dpi before stabilizing and the animal succumbs to infection 12–18dpi. A low-grade fever is detectable a few days following the detection of viremia (~6dpi) and increases for several days before falling quickly resulting in hypothermia. Weight loss is measurable a day after fever and continues until death with some animals losing >20% of total weight. Peak virus titers are detected in lymph nodes, salivary glands, spleen and lungs. Virus replication appears to be slightly lower in the liver, but lesions in the liver are apparent in both models. Tissue damage in guinea pigs is broad and changes are seen in the cardiac, hepatic and lymphoid tissues. Guinea pigs also suffer from interstitial pneumonia but all lesions are mild to moderate and do not necessarily account for lethal outcome of the infection ([3,17–19]) (Table 1). Guinea pigs that survive infection develop mild to moderate disease and can go on to develop persistent infections. The eye was recently investigated as a potential reservoir in the body. Viral antigen was found in the anterior regions of the eye in animals that succumbed but minimal damage was seen in surviving animals, consistent to what has been reported in humans [20]. Persistently infected guinea pigs develop many other sequalae associated with inflammatory conditions with long-term systemic vasculitis and periarteritis [21] that has been associated with hearing loss in other models but has not been reported in guinea pigs.

Non-human primates

NHP models of LF are considered the gold standard and the disease course closely mimics human LF. To date, LF models have been described in the squirrel monkey, the common marmoset, the rhesus macaque and the cynomolgus macaque [19,22,23]. The squirrel monkey model was the first NHP model established but its use has not continued since the first descriptive studies. The 3 remaining NHP models currently in use all reflect LF symptoms similar to humans although the marmoset has been reported to suffer uniform lethality [24]. The rhesus have survival rate of ~40% following infection with the prototypical lab Josiah strain [23,25]. The cynomolgus macaque has been the most extensively studied NHP and the severity of disease and mortality is dependent on dose and LASV strain [23,26–28].

The NHP models share important disease characteristics consistent with human LF and are sensitive to several strains of LASV (Figure 1, Table 1). Infection results in detectable viremia as early as 3dpi and increases until the time of euthanasia in terminal animals. Similar to humans, viremia is an indicator of survival and animals that do not develop a high viremia are more likely to survive infection [3,23,28,29]. The disease course in these NHPs does vary slightly by time and the macaques succumb to LF as early as 9dpi [30] but marmosets [31] do not succumb until 15dpi [24]. Infected animals can show clinical symptoms of lethargy, aphagia, constipation and low-grade fever at early times during infection, conjunctivitis and a rash may appear a few days later.

Figure 1



Experimental approaches with LASV animal disease models.

Liver enzymes (ALT and AST) become elevated following increasing viremia and continue to increase until the animal succumbs or resolves with virus clearance. Serum albumin and total protein levels fall as illness progresses and similarly rebound as the virus is cleared or the animal succumbs to disease [3,23–25,28,29].

Tissue alteration in animals that succumb to disease are consistent with limited reports in humans. Multi-focal hepatic necrosis is the most prominent lesion observed. Interstitial pneumonia of varying severity is also commonly found. Lesions are also frequently seen in the spleen, heart, varying lymph nodes and the kidneys [3,23–25,28,29]. Lesions in the CNS are less frequently seen but do occur in the brain, spinal cord and meninges [17,23,29]. These NHP models are very consistent to what has been reported on clinical LF. NHPs with LF often develop confusion, tremors, seizures or convulsions, ultimately the animal can become comatose. Interestingly, as with humans, none of the lesions detected are severe enough to account for death and animals appear to succumb from a multi-system failure (Table 1).

Mice

Wild-type mouse strains are resistant to disease following LASV infection by most routes of infection. The only infection route in adult mice resulting in disease and mortality is intracranial inoculation which does not correlate with LF observed in human [31].

However, many recent advances have been made in developing a mouse model for LASV and several immunocompromised mouse strains have been found to be sensitive to LASV infection (Figure 1, Table 1). Currently, mice deficient for type I IFN receptor ($IFNAR^{-/-}$) and type I/II IFN receptors ($IFN\text{-}a/bgR^{-/-}$) as well as the STAT1 knock out ($STAT1^{-/-}$) mice, all develop disease following LASV infection [32–38]. The $IFNAR^{-/-}$ develop clinical symptoms of ruffled fur, reduced activity and weight loss while $IFN\text{-}a/bgR^{-/-}$ mice show no signs of disease beyond weight loss. All mice lacking a functional IFN signaling develop persistent infection following LASV inoculation and virus titers are detected in the blood, brain, spleen and visceral organs ~25dpi [37,39,40] (Table 1).

$STAT1^{-/-}$ mice are highly susceptible to infection by LASV [40] and develop clinical disease exhibiting ruffled fur, weight loss and a drop in temperature with neurological signs before succumbing to disease by day 4 and day 7 pi. $STAT1^{-/-}$ mice also show generalized infection, accumulation of macrophages and an increased number of apoptotic cells in the spleen similar to what has been reported in human cases [41,42]. Interestingly, $STAT1^{-/-}$ are not susceptible to all LASV strains, infection with a LASV isolated from a lethal human case developed clinical disease by day 5 p.i. and a high rate of mortality (90%) by 8dpi. This infection was accompanied by high viral load in the blood, with brain and lymph node lesions at the time of death. In contrast, $STAT1^{-/-}$ mice infected with LASV isolated from a non-lethal human case induced disease with moderate mortality (50%) by 10dpi with the surviving mice demonstrating chronic infection [43] (Table 1).

A chimeric mouse model has been generated by transplantation of bone marrow progenitor cells from wt C57BL/6 to mouse lacking type I IFN ($IFNAR^{-/-}$). These chimeric $IFNA^{-/-B6}$ mice develop clinical diseases such as high viremia, weight loss, high level of AST and death by day 7 pi [44]. Similar to the $IFNAR^{-/-}$ and $STAT1^{-/-}$ mice, these chimeric mice develop moderate tissues damage accompanied with infiltration of granulocytes and an increase number of $CD8^+$ T cells, but not $CD4^+$ T cells, in the spleen and lung (Table 1).

A humanized mouse model genetically engineered to express a human/mouse-chimeric HLA-A2.1 molecule instead of the murine MHC class I gene products (HHD mice) [45–47] is sensitive to LASV infection.

HHD mice develop a persistent and high viral load accompanied with clinical disease such as an increase of AST activity by day 7 pi and approximately 20% of animals succumb to infection. LASV-infected HHD mice show disseminated infection, accumulation of granulocytes and T cells in the spleen, lung and liver as reported in human LF cases (Table 1).

Strategy and uses of animal models

The animal model used for studies largely depends on what question the experiment is designed to be addressing (Figure 1). Generally, the first model for most studies to consider would be a rodent, due to their small size, low cost, and the availability of immunological reagents. The mouse models can be useful to study several different aspects of LASV infection including immune responses, antivirals and vaccines. The IFNAR$^{-/-}$ model has been used successfully to examine different combinations of antivirals [48]. The STAT1$^{-/-}$ strain is a useful pathogenesis model and can be used to study chronic infection and deafness [43,49–51]. The role of T cells in LASV infection has been studied using HHD mice [47]. CD4^{+} and CD8^{+} T-cells depletion studies have indicated that viral replication does not directly correlate to tissue damage as the depleted animals do not develop tissue damage, particularly the disruption of the white and red pulp compartments in the spleen, as seen in humans [52,53]. These data implicate H-2 class I-restriction induces a T-cell mediated hepatitis in LASV infected HHD mice similar to other arenaviruses [54], supporting the hypothesis that CD4^{+} and CD8^{+} T cells may play a key role in LASV pathogenesis [55,56]. Overall, the mouse models are based on immunocompromised animals and mimic certain aspects of human LF but do not fully recapitulate the multifactorial pathophysiology of LASV infection in humans.

The guinea pig model of LASV infection shares several disease characteristics of LF in humans including fever, viral titers in the blood and tissue and visceral lesions (Figure 1, Table 1). For these reasons the guinea pig is a solid model for testing vaccine platforms or therapeutic interventions [19,57–62]. Although scientifically advantageous in these studies, guinea pigs are more cumbersome to handle than mice in BSL4 settings and require larger caging in spaces that are limited. The strain 13 guinea pigs are not commercially available and it can be difficult to acquire the appropriate numbers of age-matched animals required for a study. This problem has been partially alleviated by the recent development of the guinea pig adapted LASV used in Hartley guinea pigs. The outbred Hartley guinea pig is readily available from commercial sources but this model is only suitable for studies utilizing the Josiah strain of LASV. The guinea pig model is also hampered by the lack of specific reagents used in downstream analysis for immunology-based studies (Figure 1, Table 1).

The rhesus macaque develops a disease very similar to LF in humans and was the first NHP model of LF used to test a treatment when the effects of the antiviral drug ribavirin were assessed in a LASV Josiah infection [29]. The success of this animal study resulted in the clinical use of ribavirin to treat LF across West Africa [42]. This was followed up by examining protection acquired by the passive transfer of antibodies in survivor serum using cynomolgus macaques [63]. Marmosets are probably the least used NHP model as they are difficult to work with, at times problematic to acquire, prone to complications (i.e. ketoacidosis resulting in stress hepatitis, infection of the gastrointestinal tract), although they are a useful pathogenesis model [24] and have been used to evaluate vaccines [64]. The macaque model has become the preferred model recently and cynomolgus macaques have been used to assess vaccine efficacy, antivirals and antibody therapy [30,65–67] (Figure 1, Table 1).

Hearing impairment is common among LF survivors with an estimated 30% experiencing unilateral or bilateral hearing loss [49–51,68,69]. Although little is currently understood about LF induced hearing loss there has been progress in developing animal models to help understand how this occurs. A sensorineural hearing loss model has been developed utilizing a LASV isolate from Sierra Leone in STAT1$^{-/-}$ mice. This infection results in a milder, non-lethal infection where the animals display a permanent, reduced level of responsiveness that develops as early as D16pi. A significant degeneration was found in the spinal ganglion specific to the auditory nerve and high levels of viral antigen and CD3^{+} cells were detected along this nerve. Damage was also seen in both the outer and inner hair cells in these animals [43]. A recent report describes a similar result in the cynomolgus model of LF. Animals surviving LASV infection developed a chronic disease state and developed unilateral or bilateral hearing loss by 28dpi. Acute perivascular inflammation surrounding the cochlear nerve was reported and this chronic infection appears similar to autoimmune associated vasculitis disease that have been described in humans [28]. Very little is known about hearing loss in humans to suggest a mechanism. Both the STAT1$^{-/-}$ mouse and cynomolgus macaque models have produced mechanisms suggesting the underlying cause is based in persistent inflammatory response to the presence of viral antigen (Table 1).

Conclusions and perspective

LASV was identified 50 years ago and there is still no vaccine or FDA approved treatment available for LF. This underlies the facts that LF studies are difficult and ethically challenging. Although there has been progress, the difficulty of working in the required BSL-4 environment limits the numbers of studies that can occur. To facilitate research that is otherwise unfeasible or unethical, the FDA introduced what is known as the

Animal Rule (21 CRF 601.90, subpart H, 2007). Simply put, this rule allows the FDA to rely on evidence accumulated during animal studies to assess effectiveness of a product. This is rule is only applicable to animal studies if the model reliably replicates several disease parameters. While current LASV mouse models generally do not meet these criteria, they can be used as an important first step in screening treatments and vaccines. Mice will also continue to provide useful immunological insight into LASV infection. The guinea pig model does replicate several characteristics of LF and shares several similarities to humans both in viral kinetics and disease. The guinea pig remains a very useful tool for evaluating LASV prophylactics but does not offer the same value in terms of immunological studies as mice at this time. The NHP models, particularly the cynomolgus macaque, very closely replicate human disease and remain the gold standard for LF research (Figure 1). The value of studying LASV infection in NHPs cannot be understated when a rigorous examination of LASV infection is required. This value was first realized in the initial ribavirin studies and now continues to assist in advancing research into both vaccines and treatments. The NHP models of LASV infection fulfill the FDA Animal Rule requirements and their use should be considered in this capacity when scientifically merited (Table 1).

As LASV is a zoonotic pathogen, studying the reservoir species would likely provide extremely helpful information for virus maintenance in nature and transmission to humans. This could be done best and controlled if breeding colonies of *Mastomys natalensis* could be established for experimental studies. Those colonies would also be helpful to address the interesting concept of a wildlife vaccine for LASV to be used in the natural reservoir species.

Acknowledgements

We would like to acknowledge and thank Atsushi Okumura of the Center for Infection and Immunity at the Mailman School of Public Health, Columbia University for his helpful discussions on the laboratory use of common marmoset.

We would also like to acknowledge and thank Ryan Kissinger of Rocky Mountain Laboratories Media Arts for his illustrations in Figure 1.

Work on Lassa virus at the Laboratory of Virology has been supported by the Intramural Research Program and the International Centers for Excellence in Research (ICER) both of NIAID, NIH.

References

1. Frame JD *et al.*: **Lassa fever, a new virus disease of man from West Africa. I. Clinical description and pathological findings**. *Am J Trop Med Hyg* 1970, **19**:670-676.
2. Monath TP *et al.*: **Lassa virus isolation from *Mastomys natalensis* rodents during an epidemic in Sierra Leone**. *Science* 1974, **185**:263-265.
3. Walker DH *et al.*: **Comparative pathology of Lassa virus infection in monkeys, guinea-pigs, and *Mastomys natalensis***. *Bull World Health Organ* 1975, **52**:523-534.
4. Olayemi A *et al.*: **New Hosts of The Lassa Virus**. *Sci Rep* 2016, **6** p. 25280.
5. Bowen MD *et al.*: **Genetic diversity among Lassa virus strains**. *J Virol* 2000, **74**:6992-7004.
6. Manning JT, Forrester N, Paessler S: **Lassa virus isolates from Mali and the Ivory Coast represent an emerging fifth lineage**. *Front Microbiol* 2015, **6** p. 1037.
7. Hallam HJ *et al.*: **Baseline mapping of Lassa fever virology, epidemiology and vaccine research and development**. *NPJ Vaccines* 2018, **3** p. 11.
8. Whitmer SLM *et al.*: **New lineage of Lassa virus, Togo, 2016**. *Emerg Infect Dis* 2018, **24**:599-602.
9. Ortiz-Riano E *et al.*: **Arenavirus reverse genetics for vaccine development**. *J Gen Virol* 2013, **94**:1175-1188.
10. Martinez-Sobrido L, Paessler S, de la Torre JC: **Lassa virus reverse genetics**. *Methods Mol Biol* 2017, **1602**:185-204.
11. McCormick JB *et al.*: **Lassa fever. Effective therapy with ribavirin**. *N Engl J Med* 1986, **314**:20-26.
12. Bausch DG *et al.*: **Review of the literature and proposed guidelines for the use of oral ribavirin as postexposure prophylaxis for Lassa fever**. *Clin Infect Dis* 2010, **51**:1435-1441.
13. Ilori EA *et al.*: **Increase in Lassa fever cases in Nigeria, January-March 2018**. *Emerg Infect Dis* 2019, **25**.
14. Ehlkes L *et al.*: **Management of a Lassa fever outbreak, Rhineland-Palatinate, Germany, 2016**. *Euro Surveill* 2017, **22**.
15. Kofman A, Choi MJ, Rollin PE: **Lassa fever in travelers from West Africa, 1969-2016**. *Emerg Infect Dis* 2019, **25**:245-248.
16. Marien J *et al.*: **No measurable adverse effects of Lassa, Morogoro and Gairo arenaviruses on their rodent reservoir host in natural conditions**. *Parasit Vectors* 2017, **10**:210.
17. Jahrling PB *et al.*: **Pathogenesis of Lassa virus infection in guinea pigs**. *Infect Immun* 1982, **37**:771-778.
18. Bell TM *et al.*: **Temporal progression of lesions in guinea pigs infected with Lassa virus**. *Vet Pathol* 2017, **54**:549-562.
19. Safronetz D *et al.*: **The broad-spectrum antiviral favipiravir protects guinea pigs from lethal Lassa virus infection post-disease onset**. *Sci Rep* 2015, **5** p. 14775.
20. Gary JM, Welch RS, Ritter JM, Coleman-McCray J, Huynh T, Kainulainen MH *et al.*: **Lassa virus targeting of anterior uvea and endothelium of cornea and conjunctiva in eye of guinea pig model**. *Emerg Infect Dis* 2019, **25**.
21. Liu DX *et al.*: **Persistence of Lassa virus associated with severe systemic arteritis in convalescing guinea pigs (*Cavia porcellus*)**. *J Infect Dis* 2018.
22. Walker DH, Wulff H, Murphy FA: **Experimental Lassa virus infection in the squirrel monkey**. *Am J Pathol* 1975, **80**:261-278.
23. Hensley LE *et al.*: **Pathogenesis of Lassa fever in cynomolgus macaques**. *Virol J* 2011, **8**:205.
24. Carrion R Jr *et al.*: **Lassa virus infection in experimentally infected marmosets: liver pathology and immunophenotypic alterations in target tissues**. *J Virol* 2007, **81**:6482-6490.
25. Callis RT, Jahrling PB, DePaoli A: **Pathology of Lassa virus infection in the rhesus monkey**. *Am J Trop Med Hyg* 1982, **31**:1038-1045.
26. Safronetz D *et al.*: **A recently isolated Lassa virus from Mali demonstrates atypical clinical disease manifestations and decreased virulence in cynomolgus macaques**. *J Infect Dis* 2013, **207**:1316-1327.
27. Baize S *et al.*: **Early and strong immune responses are associated with control of viral replication and recovery in**

Lassa virus-infected cynomolgus monkeys. *J Virol* 2009, **83**:5890-5903.

28. Cashman KA *et al.*: **Immune-mediated systemic vasculitis as the proposed cause of sudden-onset sensorineural hearing loss following Lassa Virus exposure in cynomolgus macaques**. *MBio* 2018, **9**.

29. Jahrling PB *et al.*: **Lassa virus infection of rhesus monkeys: pathogenesis and treatment with ribavirin**. *J Infect Dis* 1980, **141**:580-589.

30. Rosenke K *et al.*: **Use of favipiravir to treat Lassa virus infection in macaques**. *Emerg Infect Dis* 2018, **24**:1696-1699.

31. Lukashevich IS: **Lassa virus lethality for inbred mice**. *Ann Soc Belg Med Trop* 1985, **65**:207-209.

32. Meraz MA *et al.*: **Targeted disruption of the Stat1 gene in mice reveals unexpected physiologic specificity in the JAK-STAT signaling pathway**. *Cell* 1996, **84**:431-442.

33. Hwang SY *et al.*: **A null mutation in the gene encoding a type I interferon receptor component eliminates antiproliferative and antiviral responses to interferons alpha and beta and alters macrophage responses**. *Proc Natl Acad Sci U S A* 1995, **92**:11284-11288.

34. Durbin JE *et al.*: **Targeted disruption of the mouse Stat1 gene results in compromised innate immunity to viral disease**. *Cell* 1996, **84**:443-450.

35. Sato M, Taniguchi T, Tanaka N: **The interferon system and interferon regulatory factor transcription factors – studies from gene knockout mice**. *Cytokine Growth Factor Rev* 2001, **12**:133-142.

36. Schoggins JW: **Interferon-stimulated genes: roles in viral pathogenesis**. *Curr Opin Virol* 2014, **6**:40-46.

37. van den Broek MF *et al.*: **Immune defence in mice lacking type I and/or type II interferon receptors**. *Immunol Rev* 1995, **148**:5-18.

38. Muller U *et al.*: **Functional role of type I and type II interferons in antiviral defense**. *Science* 1994, **264**:1918-1921.

39. Rieger T, Merkler D, Gunther S: **Infection of type I interferon receptor-deficient mice with various old world arenaviruses: a model for studying virulence and host species barriers**. *PLoS One* 2013, **8** p. e72290.

40. Yun NE *et al.*: **Functional interferon system is required for clearance of Lassa virus**. *J Virol* 2012, **86**:3389-3392.

41. Walker DH *et al.*: **Pathologic and virologic study of fatal Lassa fever in man**. *Am J Pathol* 1982, **107**:349-356.

42. McCormick JB *et al.*: **Lassa virus hepatitis: a study of fatal Lassa fever in humans**. *Am J Trop Med Hyg* 1986, **35**:401-407.

43. Yun NE *et al.*: **Animal model of sensorineural hearing loss associated with Lassa virus infection**. *J Virol* 2015, **90**: 2920-2927.

44. Oestereich L *et al.*: **Chimeric mice with competent hematopoietic immunity reproduce key features of severe Lassa fever**. *PLoS Pathog* 2016, **12** p. e1005656.

45. Pascolo S *et al.*: **HLA-A2.1-restricted education and cytolytic activity of CD8(+) T lymphocytes from beta2 microglobulin (beta2m) HLA-A2.1 monochain transgenic H-2Db beta2m double knockout mice**. *J Exp Med* 1997, **185**:2043-2051.

46. Arnold B, Hammerling GJ: **MHC class-I transgenic mice**. *Annu Rev Immunol* 1991, **9**:297-322.

47. Flatz L *et al.*: **T cell-dependence of Lassa fever pathogenesis**. *PLoS Pathog* 2010, **6**:e1000836.

48. Oestereich L *et al.*: **Efficacy of favipiravir alone and in combination with ribavirin in a lethal, immunocompetent mouse model of Lassa fever**. *J Infect Dis* 2016, **213**:934-938.

49. Cummins D: **Rats, fever and sudden deafness in Sierra Leone**. *Trop Doct* 1992, **22**:83-84.

50. Liao BS, Byl FM, Adour KK: **Audiometric comparison of Lassa fever hearing loss and idiopathic sudden hearing loss: evidence for viral cause**. *Otolaryngol Head Neck Surg* 1992, **106**:226-229.

51. Mateer EJ *et al.*: **Lassa fever-induced sensorineural hearing loss: a neglected public health and social burden**. *PLoS Negl Trop Dis* 2018, **12** p. e0006187.

52. Edington GM, White HA: **The pathology of Lassa fever**. *Trans R Soc Trop Med Hyg* 1972, **66**:381-389.

53. Winn WC Jr *et al.*: **Lassa virus hepatitis. Observations on a fatal case from the 1972 Sierra Leone epidemic**. *Arch Pathol* 1975, **99**:599-604.

54. Zinkernagel RM *et al.*: **T cell-mediated hepatitis in mice infected with lymphocytic choriomeningitis virus. Liver cell destruction by H-2 class I-restricted virus-specific cytotoxic T cells as a physiological correlate of the 51Cr-release assay?** *J Exp Med* 1986, **164**:1075-1092.

55. Goicochea MA *et al.*: **Evaluation of Lassa virus vaccine immunogenicity in a CBA/J-ML29 mouse model**. *Vaccine* 2012, **30**:1445-1452.

56. Mongkolsapaya J *et al.*: **Original antigenic sin and apoptosis in the pathogenesis of dengue hemorrhagic fever**. *Nat Med* 2003, **9**:921-927.

57. Cross RW *et al.*: **Treatment of Lassa virus infection in outbred guinea pigs with first-in-class human monoclonal antibodies**. *Antiviral Res* 2016, **133**:218-222.

58. Kainulainen MH *et al.*: **Use of a scalable replicon-particle vaccine to protect against lethal Lassa virus infection in the guinea pig model**. *J Infect Dis* 2018, **217**:1957-1966.

59. Morrison HG *et al.*: **Protection of guinea pigs from Lassa fever by vaccinia virus recombinants expressing the nucleoprotein or the envelope glycoproteins of Lassa virus**. *Virology* 1989, **171**:179-188.

60. Safronetz D *et al.*: **A recombinant vesicular stomatitis virus-based Lassa fever vaccine protects guinea pigs and macaques against challenge with geographically and genetically distinct Lassa viruses**. *PLoS Negl Trop Dis* 2015, **9** p. e0003736.

61. Cashman KA *et al.*: **DNA vaccines elicit durable protective immunity against individual or simultaneous infections with Lassa and Ebola viruses in guinea pigs**. *Hum Vaccin Immunother* 2017, **13**:3010-3019.

62. Carrion R Jr *et al.*: **A ML29 reassortant virus protects guinea pigs against a distantly related Nigerian strain of Lassa virus and can provide sterilizing immunity**. *Vaccine* 2007, **25**: 4093-4102.

63. Jahrling PB, Peters CJ: **Passive antibody therapy of Lassa fever in cynomolgus monkeys: importance of neutralizing antibody and Lassa virus strain**. *Infect Immun* 1984, **44**:528-533.

64. Lukashevich IS *et al.*: **Safety, immunogenicity, and efficacy of the ML29 reassortant vaccine for Lassa fever in small non-human primates**. *Vaccine* 2008, **26**:5246-5254.

65. Cashman KA *et al.*: **A DNA vaccine delivered by dermal electroporation fully protects cynomolgus macaques against Lassa fever**. *Hum Vaccin Immunother* 2017, **13**:2902-2911.

66. Mire CE *et al.*: **Human-monoclonal-antibody therapy protects nonhuman primates against advanced Lassa fever**. *Nat Med* 2017, **23**:1146-1149.

67. Geisbert TW *et al.*: **Development of a new vaccine for the prevention of Lassa fever**. *PLoS Med* 2005, **2** p. e183.

68. Okokhere PO, Ibekwe TS, Akpede GO: **Sensorineural hearing loss in Lassa fever: two case reports**. *J Med Case Rep* 2009, **3** p. 36.

69. Simmons FB: **Lassa fever and sudden hearing loss**. *Otolaryngol Head Neck Surg* 1993, **109** p. 559.