Chapter

Applications of Animal Models in Researching Hepatitis A

Huafeng Lin, Aiping Min, Gang Li, Yan Lei Chang, Lei Shi and Dan Qiu

Abstract

Hepatitis diseases are remaining in the list of significant threats to human health. Human hepatitis viruses are basically classified into six major hepatotropic pathogens—hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV), hepatitis E virus (HEV), and hepatitis G virus (HGV). Among these different forms of hepatotropic viruses, HAV as the leading cause of acute viral hepatitis is characterized as a kind of tiny ribonucleic acid virus that is linked to atopic disease. As we know, animal models have been instrumental in promoting understanding of complex host-virus interactions and boosting the advancement of immune therapies. So far, animal models such as nonhuman primates (NHPs) have enabled scientists to mimic and study the pathogenicities and host immune responses for hepatitis A infection. With the exception of chimpanzees and marmosets, animals like mice, pigs, guinea pigs, and tree shrews can also be selected as alternative animal models infected with HAV under laboratory conditions. In order to gain a better insight into hepatitis A pathogenesis and relevant contents, this chapter is mainly focused on the research progress in animal models of hepatitis A, and discusses the merits and demerits of these alternative models.

Keywords: hepatitis A, infection experiments, animal models, virus hepatitis

1. Introduction

Various forms of viral hepatitis represent a world health concern and challenge, generating a considerable socio-economic burden. Of these, hepatitis A as a type of food-borne hepatitis is mainly endemic in developing regions with the condition of inadequate sanitation and hygiene, such as in parts of Africa, Asia, Eastern Europe, South America, and Middle East [1, 2]. With the improvement of public health, the incidence of HAV infections in China have been gradually reduced (published data from 1990 to 2017) [3]. Up to now, 1.5 million cases of hepatitis A virus (HAV) infections are reported worldwide [2], which indicated that hepatitis A remains a primary problem in hygiene and public health. Hepatitis A has a very similar clinical symptom compared to hepatitis E. Except for the severer pathological injuries of hepatitis E than that of hepatitis A, both of two are self-limiting diseases, do not lead to liver cirrhosis and liver cancer, and transmit via orofecal route and person-to-person contact [4]. Consequently, HAV-contaminated water, vegetables, fruits, blood products, and other foodstuffs, especially undercooked shellfish including clams, oysters, and mussels (**Figure 1**) [5, 6], are the major pathways of infections

Figure 1.
Diagram showing the possible transmission routes of HAV.

with hepatitis A [7, 8]. Under certain circumstances, intravenous drug users with the collective use of syringes are at risk categories for HAV infections [9], and also there exist vertical transmissions of HAV from mother to child but occur very rarely (**Figure 1**) [10]. HAV as the main pathogen causing acute viral hepatitis is classified as a sole member of the genus Hepatovirus of the family *Picornaviridae*, which includes many medical and veterinary pathogens in 1991 [11–13]. HAV is a single linear positive-stranded RNA virus whose genomic full length is approximately 7500 nucleotides, which contain 5′-noncoding region (UTR), protein coding region, and 3′-noncoding region (UTR) (**Figure 2**) [13]. Researchers have found that HAV present in the form of naked, nonenveloped virions in feces aids to the viral transmissions through the environment. However, when HAV emerges in the blood of infected persons, the virion isolates itself from neutralizing antibodies by the way of producing quasi-envelope in host-derived membranes [14]. Epidemiological data showed that the most susceptible populations of HAV are the children in early childhood [2], and the disease prevalence exceeds 90% before the age of 10 [15], albeit most of infected youngers are usually mild or asymptomatic [16]. Hence, accelerating the immunological research and viral vaccine development can improve human immunity and reduce the spread of HAV. World Health Organization (WHO) recommends that vaccination combating HAV be integrated into the national immunization schedule for children aged ≥1 year on the consideration of many factors including cost-effectiveness [17]. What is noteworthiness is that, the illness infected with HAV in those people who are older than 60 will be very severe [18]. Moreover, HAV superinfections in chronic liver disease (CLD)

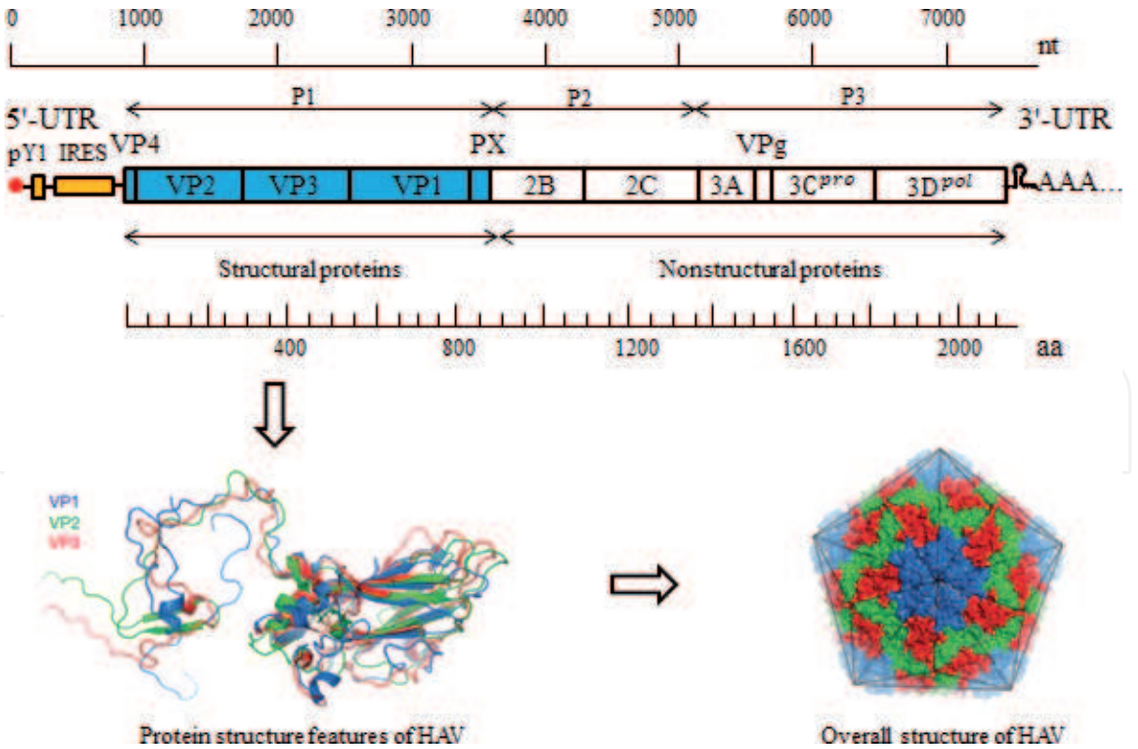

Figure 2.
The genome structure, protein structure components and overall structure of HAV. Refer to [28, 35, 36].

sufferers (e.g., hepatitis B or C) are usually associated with raising morbidity and mortality [19, 20]. To date, animal model is one of the promising tools in the investigation of human HAV infections. Studies on HAV immunopathological mechanism and host immune response mainly used nonhuman primates such as chimpanzees and marmosets as animal models. Due to the lack of other alternative animal models that support HAV infections, the study of the HAV biology and further development of therapies for hepatitis A have been hampered. Here in this chapter, the biological features of HAV will be discussed, the animal models of hepatitis A and their characteristics will be sketched, and the merits and demerits for these models will be analyzed as well.

2. Basic biological features of HAV and beyond

As early as 5000 years ago, hepatitis A-like illnesses were documented in ancient China. In Europe, similar disease known as "benign epidemic jaundice" was also described during the Hippocratic era [21]. As time goes by, in 1947, McCallum et al. termed infectious hepatitis as hepatitis A [22]. In the first half of 1967, Krugman et al. found the distinctive features between infectious hepatitis (hepatitis A) and serum hepatitis (hepatitis B) in clinical, epidemiological, and immunological aspects [23]. By 1973, Feinslone et al. firstly detected hepatitis A in feces of patients using the technology of immune electron microscopy (IEM) [24]. Morphologically and structurally, the purified HAV virion, having an outer diameter of 27–80 nm, is an icosahedral nucleocapsid protein granular which contains one linear positive-stranded ribonucleic acid (RNA) genome [25]. The genome encodes a single large polyprotein of 2227 amino acids [26], which is matured and folded to produce 10 biologically active viral proteins, including four structural proteins that construct the capsid (VP4 (~2.5 kDa), VP2 (24–30 kDa), VP3 (21–28 kDa), and VP1pX) and 6 nonstructural proteins that are indispensable for replication of the RNA genome (2B, 2C, 3A, 3B [VPg], $3C^{pro}$ [a cysteine protease], and $3D^{pol}$) (RNA-dependent

RNA polymerase) (**Figure 2**) [27]. By using standard serological technique and molecular identification methods, HAV is identified to belong to merely one single serotype, and is divided into seven distinct genotypes of which three genotypes (I, II, and VII) that circulate in humans, one genotype (III) isolated from either humans or owl monkeys, and other three genotypes (V–VII) exist in nonhuman primates [28–30]. Genotypes I, II, and III are sub-classified into subtypes A and B (Genotypes IA, IB, IIA, IIB, IIIA, and IIIB) [31]. Molecular epidemiology has further revealed that HAV sub-genotype IA is responsible for the most circulations among human population [32]. For sub-genotype IB HAV strains, several reports have declared that they were associated with food such as frozen strawberries in Australia and several countries of Europe [33, 34]. Recent studies of X-ray analysis have uncovered that HAV possesses a primitive capsid architecture related to that of picorna-like viruses infecting insects, which imply a correlation of primeval evolution as well as a novel cellular entrance mechanism for viruses [35]. The structure information (especially the 3D microstructural study) of viral protein is now a robust tool for dissecting their biological functions. In 2018, Stuart et al. reviewed updated studies on the structural features of outer protein shell of HAV and proposed the future researching scopes including relevant structural elucidations of the enveloped particles, as well as the capture of intermediates in the state of assembly, attachment, and/or uncoating [36]. In terms of receptor binding mechanism, Wang et al. pointed out that using a receptor mimic mechanism for neutralization of infectivity may hold promise for the therapeutic intervention of hepatitis A [37]. With regard to the origin of human HAV, phylogenetic analyses show that, in the remote past, these ancient viruses have emerged in different host species, and ancestral state reconstructions indicate HAV is likely to have originated in rodents [38]. What's more, investigations should be fundamentally focused on therapeutic interventions and new creations of HAV vaccines as a result of hepatitis A vaccine is one of the most effective strategy for the treatment of hepatitis A [39]. To date, four inactivated monovalent HAV vaccines from different manufactures (Havrix®, Epaxal®, Avaxim®, and Vaqta®) have been commercially available to the global markets [40]. Other hepatitis A vaccines such as a Chinese live attenuated vaccine (H2 strain, Zhejiang Academy of Medical Sciences, Hangzhou, PR China) and a Vietnam one are just self-sufficient in domestic production [41]. HAV infections are still an important cause of morbidity and mortality in developed countries such as the United States [42], let alone other nations with poor sanitation. Therefore, the work of scientific research for hepatitis A vaccine is still certainly on the way.

3. Applications of animal models

According to literatures, HAV strains of wild type are quite difficult in propagating in vitro. When culturing in cell-conditioned medium, they show low growth rate characteristically, as well as have no apparent cytopathic effects [43]. Additionally, HAV has its own special life history: it primarily replicates in the hepatic tissue, is excreted in biliary system to reach the intestinal contents [44], and is mostly shed in the feces and soil [45], where the viruses may persist for an extended period of time [46, 47]. Consequently, it is significantly important for researchers to find the proper infected models that aim at the investigation of HAV. As Hirai-Yuki and his co-authors have ever pointed out that, it is essential to develop improved animal models for the deeper investigations of the molecular and cellular mechanisms associated virus-hepatocyte interactions within the distinctive environment of liver tissue of hosts [48]. Here are the examples of disease models for HAV infections showed in **Table 1**.

Authors/year	Animal models	Comments	Refs
Dienstag et al/1975	Chimpanzees	Provided evidence for the susceptibility of chimpanzees to HAV	[49]
Amado et al/2010	Cynomolgus monkeys	Cynomolgus monkey was confirmed as a suitable model to study HAV infection	[67]
LeDuc et al/1983	New World owl monkeys	Confirmed the susceptibility of New World owl monkey to HAV	[53]
Song et al/2016	Pigs	First experimental evidence to demonstrate human HAV strains can infect pigs	[76]
Hirai-Yuki et al/2016	Mice	Provided a new paradigm for viral pathogenesis in the liver	[83]
Hornei et al/2001	Guinea pigs	Useful for studying some aspects of HAV pathogenesis and for testing the safety of vaccines.	[88]
Zhan et al/1981	Tree shrews	HAV can replicate in tree shrews and are potential for candidate models for HAV infections	[97]
Anthony et al/2015	Harbor seals	Describe the discovery of an HAV-like virus in seals	[98]
Liu et al/2019	Pekin ducks	There are differences in the pathogenicity of different subtypes of DHAV in ducklings	[100]
Wen et al/2019	Ducks	Provided new insights into the genetics and pathogenesis of DHAV-3	[101]

Table 1.
Examples of disease models for HAV infections.

3.1 NHPs

Broadly speaking, NHPs resemble humans in anatomy, physiology, and pathology over any other animals, which make them to be considered as the principal models for diseases including HAV infections. Human HAV has successfully infected various species of NHPs, such as chimpanzees (*Pan troglodytes*) [49], common marmosets (*Callithrix jacchus*) [50, 51], Squirrel Monkeys (*Saimiri sciureus*) [52], New World owl monkeys (*Platyrrhines*) [53], African green monkeys (*Cercopithecus aethiops*) [54], owl monkey (*Aotus trivirgatus*) [55], brown macaques (*Macaca arctoides*) [56], stump-tailed monkey (*Macaca speciosa*) [57] and tamarins, etc. (**Table 1**), but the host range of this virus is still narrow [58], mainly limited to relatively few species. The most common animal models used in laboratories for interrogating HAV infection are mainly marmosets and chimpanzees, which are of scarce resources (Chimpanzees are so expensive that they are not widely available for research use) in most countries. In addition, experimental data indicated that more than 90% of wild chimpanzees carried anti-HAV antibodies [59], which made them less suitable for investigating HAV-infectious diseases, but chimpanzees reared in captivity are more susceptible to infect hepatitis A. Moreover, it is very difficult for laboratory technicians to feed and operate experimentally on these two animals in many situations. And quite importantly, ethical concerns have advocated the decreasing use of chimpanzees for invasive experiments of research [60].

Take chimpanzees for example, they are the candidate experimental subjects that are most closely related to humans genetically, and most probably to be simulative and predictive of human outcomes when used as disease models. In 1962, Deinhardt et al. launched the initial attemption experiment that used chimpanzees to be infected with HAV through inoculating viral materials (serum or feces), but

the gained results could not provide conclusive evidences for the transmission of infective hepatitis from humans to chimpanzees [61]. Intriguingly, in 1963, Hillis presented biochemical and histologic evidences that promisingly proved chimpanzees as useful as experimental hosts for human hepatitis viruses [62, 63]. In the mid-1970s, results of most of numerous publishment, which attempt to spread hepatitis A to chimpanzees yielded negative or equivocal results [64]. By 1984, Tsiquaye et al. carried out a study on acute hepatitis A infection occurred in hepatitis B chimpanzee carriers, which showed that superinfection can significantly alter the parameters of HBV chronicity in chimpanzees [65]. The authors pointed out that further observations were needed to establish the degree of severity of concurrent infection of HBV carriers with HAV, since such changes may have implications in some countries where the proportion of HBV carriers is high plus hepatitis A is highly prevalent [65]. For the purpose to locate where does the HAV might duplicate in the body, in 1989, Cohen and his colleagues conducted a study of single chimpanzee involvement in experiment, and found a possible oropharyngeal site for viral replication due to the emergence of HAV in saliva and throat swabs [66]. Similarly, Amado and co-authors acquired an experimental result that salivary gland was an extrahepatic site for early HAV replication in cynomolgus monkeys [67]. In the following two decades, the investigators shifted the focus of animal models to other NHPs instead of chimpanzees either because of the high cost of chimpanzee research or because of the poor contribution of chimpanzee experiments for biomedical applications [68]. Until 2011, Lanford et al. utilized three chimpanzee models to study the early innate immune responses to HAV infections. They found that HAV has a better property of keeping itself latent compared to HCV during early stage of acute resolving infection, and HAV infections represent a distinctly different paradigm in the course of intrahepatic interactions of virus-host [69].

The chimpanzees have been demonstrated to be an invaluable model tools for the investigation on HBV-induced disease pathogenesis and the discovery of novel prophylactic drugs and anti-viral therapies [70]. Optimistically, with the advancement of biotechnology, utilizing chimpanzee and other NHPs as disease models for HAV infection will surely play significant roles in HAV-associated studies in the future.

3.2 Pigs

Compared with NHPs, pigs have several advantages such as easy breeding and rearing, convenient handling and fewer ethical concerns, which make them be widely used in biomedical research [71]. Under natural conditions, it had been reported that HAV infections are being restricted to humans and nonhuman primates [72], and the appropriate models used for HAV infection have been restricted to nonhuman primates [73]. Due to several limitations of such animal models, other surrogate models need to be developed for further study. According to literatures, the immune system in pigs shares many similarities with humans for over 80% of analytical parameters, which made swine as a more suitable and common animal model for humans [74, 75]. Moreover, pigs have been used preclinically as disease models for preclinical studies usually. Until 2016, Song et al. firstly found the experiment evidence to prove human HAV strains can also infect swine [76], which took the first step to approach swine models for HAV infections. In this experiment, Song and colleagues observed that HAV can survive and replicate in pigs, which have replaced NHPs. However, there were no significant changes in the clinical manifestations and serum markers for pigs infected with HAV. Finally, they further suggested that pigs might be a suitable animal model for future studies related to HAV pathogenesis [76].

3.3 Chimeric mice/gene knock-out mice

Over the last two decades, mouse models have been successfully used in tackling various biological questions associated with intrahepatic immune response mechanisms for disease pathogenesis and clearing of HBV [77]. And also, such types of models can be applied to study the adaptive immune response to hepacivirus infection and will play roles in vaccine development. However, HAV is not capable to replicate in mice due to incompatibilities in the interaction of the virus and the innate immune system of mice. Therefore, scientists tackle this difficulty by utilizing chimeric mice, which facilitated the successful replication of HAV in the body through bypassing the cytosolic pattern recognition receptor, MAVS [78].

Generally speaking, certain cellular receptors are the key molecules that mediate viruses of entry into special kinds of cells in the body. Human membrane protein TIM-1 (T cell immunoglobulin and mucin domain protein-1) is a type of phosphatidylserine receptor that was firstly described as HAVCR1 [79], which helps cellular entry and infection with innumerable conventional enveloped viruses that bind phosphatidylserine on their surface [80]. And specially, TIM-3 receptor facilitates HAV for its entrance into target cells in humans [81]. However, recent research showed that TIM-1 is not an essential hepatovirus factor although its PtdSer-binding activity may contribute to the spread of quasi-enveloped virus and liver damage in mice [82]. For most of mouse models, wild-type mice are naturally resistant to HAV infection [83], and murine cell lines still exist defects in viral entry processes functionally [84]. For these reasons, multiple approaches have been developed by investigators to generate "humanize" mice at a genetic level to aid them susceptible to infection with HAV.

Previously, Yang et al. reported that, by using cell culture method, HAV ablates type 1 IFN responses thereby disrupting activation of IRF3 through the MDA5 pathway [85]. In 2013, Pang used HAV to infect SCID-beige/Alb-uPA mice with chimeric human/mouse livers for the purpose to test the susceptibility of mice to HAV. The result shows that these chimeric mice are permissive to HAV infection and represent valuable small animal models for future studies [86]. In 2016, Hirai-Yuki et al. applied the murine models with genetic defects in the induction of type I interferon (IFN) responses for HAV infection to reveal a previously undefined link between innate immune responses to virus infection and acute liver injury, which furnishes a novel paradigm for viral etiopathogenesis in the liver [83]. In 2018, a research team of Hirai-Yuki wrote a review of the study on "Murine models of hepatitis A virus infection" in which they provided an extensive and in-depth perspective into the development and application of mice models for HAV [48]. Additionally, in this chapter, it emphatically introduced the mechanism of degrading MAVS via viral proteases, in which it facilitates long-term survival of virus and spread through escaping from IFN-mediated restriction of virus replication and limiting pathogenesis and hepatic damage [48].

Till now, mouse models have been applied to support infections with HBV, HCV, and even HAV successfully. This probably has to do with building infections in the mouse liver, which is a key point in the development of viral hepatitis. Predictably, it has a promising future for utilizing mice as effective models for the investigation of HAV infection with the technological development of biomedical models.

3.4 Guinea pigs

The guinea pig models are more similar to humans than other small animal models in physiology and immunology. Specifically, the guinea pigs have the

property of being analogous to humans in reproductive physiology and estrous cycle [87], etc. Guinea pigs have been used as an HAV infection model, but their use is limited because of the lack of development of anti-HAV antibodies in inoculated guinea pigs. In 2001, Hornei et al. conducted a study to determine whether HAV is capable to infect guinea pigs and whether they can be valid as a disease model for replicating HAV pathogenesis in humans and for the evaluation of vaccines [88]. The authors found that very low levels of HAV were detected in the livers of guinea pigs, which inoculated with human HAV [88]. Furthermore, they also described that the experimental guinea pigs shared similar response pattern with a New World nonhuman primate (*Callithrix jacchus*) after being challenged with HAV materials [88, 89]. The method of using guinea pigs to establish models for HAV infection is still under controversy. In 2010, de Castro Araujo and colleagues designed a research project to respond to the question "Whether HAV is capable to infect guinea pigs?". However, they failed to establish a guinea pig as model for HAV [90].

3.5 Tree shrews

Chinese tree shrew (*Tupaia belangeri chinensis*), mainly distributing in Yunnan and Guangxi provinces in China, is a class of small animals having a closer evolutionary relationship with humans compared to rodents [91]. Tree shrews have emerged in the vision of scientists for more than 30 years as a result of having many valuable features that are suitable in animals utilized as experimental models in biomedical studies [92], particularly in the fields of toxicology and virology [93]. To date, there are many attempts to employ tree shrews as models for human disorders such as hepatitis B [94] and hepatitis C infections [95, 96]. In 1981, Zhan et al. used fecal suspension of hepatitis A patients (concentration: 5%) to infect nine tree shrews through oral route, and eventually no apparent clinical symptoms of acute hepatitis were found. About 7–13 days after the viral infections, seven tree shrews were detected HAV that lies in their stools in 12–27 days, which indicated that HAV could reproduce in the body of tree shrews. The experimental results indicated that HAV can replicate in tree shrews and are potential for candidate models for HAV infections [97]. Additionally, they also found disease symptoms including increased alanine transaminase (ALT), hepatic hyperemia, hepatic edema, steatosis, and hyperplasia of Kupffer cells in the infected tree shrews, which further manifested that HAV can propagate in tree shrews [97].

3.6 Other animal models

Early studies suggested that HAV was unable to lead to infections of any common small laboratory animals successfully except NHPs. However, this "prejudice" has already been challenged and overturned by animal model engineering as well as by new scientific discoveries. In 2015, several strains of human HAV have been found in seals, which may indicate that the first natural nonprimate HAV to be discovered, and provide further understanding for the evolutionary history and pathogenicity of HAV [98]. Moreover, in recent years, HAV-associated hepatoviruses have been found in bats, rodents, hedgehogs [38], duck [99–101], and woodchucks [102, 103], which suggested that there may be more candidate animals potentially used as animal models of HAV. On the contrary, some scholars believed that these new viruses are substantially more divergent from each other and from human HAV (including simian HAV), which is in accordance with them being assigned to several additional species in taxonomy [78].

4. Ethical aspects

The animal experiments definitely play an important role in the development of life sciences and medical sciences. Therefore, ethical analysis concerning animal experiments is essential because it cannot completely avoid the use of animals [104] in the process of biomedicine and preclinical medicine research. Specially, NHPs act as the particularly valuable models for testing interventions against the Ebola and Marburg viruses in the field of studying of current infectious diseases, which can effectively objectively simulate human diseases via infections in these animals [105], and further contribute to the development of new protective and therapeutic vaccines. At a certain level, ethical issues become more important than scientific interest for this type of animal test [104] because such infections are often lethal to the experimental animals, which are commonly viewed as unethical. Similarly, experiments with HAV infection also expose animals (mainly NHPs) to injury or disease. Consequently, how to balance the contradiction between ethical challenges and NHPs infectious experiments becomes a vitally important subject.

5. Future directions

5.1 Animal model methods

Animal model research is entering a new and exciting stage along with the technologies of computational information and molecular biochemistry. For example, it is now possible for us to employ genome-edited techniques (e.g., ZFNs, TALENs, and CRISPR/Cas) to knockout specific genes, to knock in new genes, or to introduce specific mutations, and then to produce valuable animal models that benefited for our investigations. However, as we know, "no model is perfect, but many are useful" [106]. Therefore, establishing susceptible animal models is one of the methods in the research fields of HAV. By using appropriate and reliable animal models, virologist can perform a series of studies associated with hepatitis A including epidemiologic features, viral infectivity, humoral and cellular immunity, cytokine responses, virus pathogenesis, as well as the research and development of antiviral vaccinations.

5.2 Cell culture methods

For the development of hepatitis A vaccines, it is worth mentioning that a highly effective vaccine against HAV was manufactured by classical inactivation of the whole virus generated from cell culture [107], which commendably avoids the ethical controversy of using NHPs models. Moreover, there is a need to provide more support for the studies of long-term protection vaccines against hepatitis A infection [108].

Last but not least, it is very likely that a much wider host range of HAV-associated viruses will be discovered in other mammalian species in the future [38].

6. Conclusion

The Nobel laureate Peter Medawar have ever succinctly concluded that "No virus is known to do good" [109]. However, as we all know, "viruses are not omnipotent." For hepatitis viruses, the narrow hepatic tissue tropism maybe is the cause

of constraining the host ranges of hepatitis viruses to relatively few special host species. As previously reported, only one serotype of HAV had been found globally [110]. However, according to Bosch et al., there exists the possibility of emergence of a novel serotype originated from zoonotic reservoirs [18]. In summary, it is necessary to further develop candidate animal models for hepatitis A infection although HAV is easily capable of adapting growth in the condition of conventional mammalian cell cultures [92].

In recent decades, HAV has been ignored by viral research circles to a certain extent due to the research spending and interest have shifted to other hepatotropic pathogens. Finally, animal model research, as a preclinical study aiming to hepatitis A, can offer a scientific platform to accelerate the pace for drugs screening and vaccines development.

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Conflict of interest

No conflicts of interest were reported.

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Acronyms and abbreviations

ALT	alanine transaminase
CLD	chronic liver disease
DHAV	duck hepatitis A virus
HAV	hepatitis A virus
HBV	hepatitis B virus
HCV	hepatitis C virus
HDV	hepatitis D virus
HEV	hepatitis E virus
HGV	hepatitis G virus
IEM	immune electron microscopy
IFN	type I interferon
IRES	internal ribosomal entry site
NHPs	nonhuman primates

TALENs	transcription activator-like effector nucleases
WHO	World Health Organization
ZFNs	zinc finger nucleases

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