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# 57 Animal Models in Virology

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## ABSTRACT

The knowledge we have gained from the study of many diseases that affect humans comes from the study of disease processes in different animal species, and this has enhanced our understanding of the pathogenesis of the disease in humans. The American Medical Association says almost every advance in medical science in the twentieth century, from antibiotics and vaccines to antidepressant drugs and organ transplants, has been achieved either directly or indirectly through the use of animals as models of disease. In this chapter a brief overview of the uses of animal models for research on human viral diseases is presented.

**Key Words:** AIDS, Animal models, Cell biochemistry, Cell-based assays, Cervical cancer, Comparative medicine, Ebola virus, Genetic alterations, Genetic make-up, Hantavirus, Hepatitis C, Human papilloma virus, Influenza virus, Intracellular parasites, Mimicry, Nipah virus, Nonhuman primates, SARS, Transgenic mice.

## INTRODUCTION

Animal models are used in almost every field of biomedical research. Almost all advances in medical knowledge and treatment, especially those in the past century, has involved work with laboratory animals. Two-thirds of the Nobel prizes awarded since 1901 have been for discoveries requiring the use of laboratory animals.

**DEFINITION OF VIROLOGY** Viruses are small infectious obligate intracellular molecular parasites. The most basic definition is that viruses are composed of a genome and one or more proteins coating that genome.

The simplest definition of virology is the study of viruses and viral diseases. What then is a virus? Viruses are small infectious obligate intracellular molecular parasites. Today the most basic definition is that viruses are composed of a genome and one or more proteins coating that genome. The virus genome is composed of either DNA or RNA. The genetic information for such a protein coat and other information required for replication are encoded in that genome. The HIV virus is a good example (human immunodeficiency virus/structure).<sup>1,2</sup> Virology is a work in progress. With much left to learn even about extensively studied

viruses, we are also certain to be challenged by new, emerging viruses.<sup>3,4</sup> At first, virologists focused on the essential interactions of viruses with cultured cells in which they are grown. However, the strategies of viruses are often focused on interactions with systems of the host animal, notably the immune system. Present and future research in virology involves unraveling the many details of the virus–host relationship. We are likely to be frustrated over and over by the subtlety of the mechanisms that have evolved both to replicate viruses and to defend against host responses. In some cases we will see new examples of viral mimicry of cellular functions. Meanwhile, intracellular locations of viral activities and associations of viral proteins and nucleic acids with cellular counterparts will continue to illuminate details of cellular organization and regulation. Animal models of many human viral diseases are still needed to help elucidate the paths of viral dissemination and specific interactions of certain cells or organs with viruses.

## SPECIFIC ROLES ANIMALS HAVE PLAYED IN RESEARCH IN HUMAN MEDICAL VIROLOGY

There are several examples of specific roles animals have played in research in virology; one of the best examples is the virus that causes AIDS. The scientific literature is filled with numerous examples of the specific roles animals have played in viral research both in humans and animals.<sup>5–8</sup> Animal models are essential for investigating and studying diseases in humans and that includes viral diseases. In the very early 1980s, when reports of a lethal immunodeficiency disease began circulating, the news at first generated deep fears because no one knew what was causing the disease. The causative agent is a retrovirus, human immunodeficiency virus (HIV).<sup>9</sup> In fact, the reverse transcriptase in the viral particle provides the signal that was used to isolate the virus for the first time. Tremendous advances in our understanding of HIV have been made through the use of animal models.<sup>9–13</sup>

While there are several examples of specific roles animals have played in research in virology, one of the best examples is the virus that causes AIDS. However, there are limitations inherent in many of the current models, whether they are nonhuman primates or other models such as feline immunodeficiency virus (FIV). An exhausting review of the literature clearly shows that while there is no ideal model for HIV, the accumulating knowledge obtained from these models have made major contributions to understanding the pathogenesis of HIV.<sup>14,15</sup> As stated by Robert Gallo, “With animals, we may have a cure for AIDS, without animals we will never cure AIDS in my lifetime.”<sup>16</sup>

Scientists are also using a number of different animal models to obtain information that can have application to HIV. FIV, transgenic mice, and rats that contain part of the HIV genome or coreceptors for viral entry, and severe combined immune deficiency (SCID) mice reconstituted with human immune system cells or tissues are some of the animal models being used to study the pathogenesis of HIV.<sup>17–19</sup>

Other examples of specific roles of animals in viral are the coronavirus, severe acute respiratory syndrome (SARS), in which the ferret and domesticated cat are susceptible to infection by SARS.<sup>20,21</sup> The scientific literature is filled with numerous examples of the specific roles animals have played in research in virology.<sup>22–24</sup>

## HISTORICAL PROSPECTIVE OF ANIMAL MODELS IN VIROLOGY

Viruses have historically provided and continue to provide the basis for much of our knowledge and understanding of modern biology, genetics, and medicine. An understanding of viral genomes and viral replication provides basic information concerning cellular processes in general.

**HISTORY OF RESEARCH ON SELECTIVE VIRAL DISEASES** Virology's history began over a century ago; viruses, including bacteriophage, have been the source for much of today's molecular biology. In the past 40 years of research on animal virology, we have watched the field go from just growing and identifying animal viruses to a highly sophisticated branch of science. In fact, viruses, including bacteriophage, are the source for much of today's molecular biology. This personal and, of necessity, somewhat limited record reflects how far virology has come and where it still needs to go.

The early definition of a virus was any infectious agent that passed through a filter that caught bacteria. Nonetheless, as early as 1927, visionaries such as Hermann Muller recognized that the small size of viruses had profound implications. There simply was not enough room inside a virus for much more than its genetic material. Of course, no one then knew what genetic material was.

Most studies in virology until the 1950s focused on laboratory animals such as mice, chickens, and ferrets. A pathfinding exception was the work of John Enders and his colleagues at Harvard Medical School who, in 1949, adapted poliovirus to grow in cell culture. This achievement not only revolutionized the production of vaccines, but it set the path for the biochemical analysis of this and other viruses. Virology and immunology—those twins of offense and defense—are generally in a fine balance, but the story of HIV shows that the balance can be tipped against the immune system by a particularly cunning agent. Under the leadership of Dr. Robert Gallo, the Institute of Human Virology is dedicated to the discovery, research, treatment, and prevention of chronic viral diseases, including HIV/AIDS.<sup>16</sup>

The study of viruses has historically provided and continues to provide the basis for much of our most fundamental understanding of modern biology, genetics, and medicine. Virology has

had an impact on the study of biological macromolecules, processes of cellular gene expression, mechanisms for generating genetic diversity, processes involved in the control of cell growth and development, aspects of molecular evolution, the mechanism of disease and response of the host to it, and the spread of disease in populations. In essence, viruses are collections of genetic information directed toward one end: their own replication. The viral genome contains the “blueprints” for virus replication enciphered in the genetic code, and must be decoded by the molecular machinery of the cell that it infects to gain this end. Viruses are obligate intracellular parasites dependent on the metabolic and genetic functions of living cells. The replication and propagation of a given virus in a population are frequently (but not always) manifest with the occurrence of an infectious disease that spreads between individuals.

The historic reason for the discovery and characterization of viruses, and a continuing major reason for their detailed study, involves the desire to understand and control the diseases and attending degrees of economic and individual distress caused by them. As science progressed, it became clear that there were many other important reasons for the study of viruses and their replication. Since viruses are parasitic on the molecular processes of gene expression and its regulation in the host cell, an understanding of viral genomes and virus replication provides basic information concerning cellular processes in general (Table 57–1).

There is archeological evidence in Egyptian mummies and medical texts of readily identifiable viral infections, including genital papillomas (warts) and poliomyelitis. There are also somewhat imperfect historical records of viral disease affecting human populations in classical and medieval times. While the recent campaign to eradicate smallpox has been successful and it no longer exists in the human population (owing to the effectiveness of vaccines against it, the genetic stability of the virus, and a well-orchestrated political and social effort to carry out the eradication), the disease periodically wreaked havoc and had profound effects on human history over thousands of years. Smallpox epidemics during the Middle Ages and later in Europe resulted in significant population losses as well as changes in the economic, religious, political, and social life of individuals. The effectiveness of vaccination strategies gradually led to the decline of the disease in other parts of the world until after World War II. Recently fears have arisen that the high virulence of the virus and its mode of spread might make it an attractive agent for bioterrorism.<sup>24</sup>

Depending on the infection and the focus of study, other animals have proven to be useful in infectious disease research. These animals include the rabbit, rat, guinea pig, pig, dog, and monkey. The latter, in particular, has been utilized in the study of AIDS, as primates are the genetically closest relatives to humans. The advent of molecular techniques of genetic alteration has made the development of genetically tailored animal models possible. Thus, for example, mouse models exist in which the activity of certain genes has been curtailed. These are known as transgenic animals. The involvement of the gene product in the infectious process is possible on a scale not possible without the use of the animal.<sup>25</sup>

**Table 57-1**  
**Human diseases caused by viruses**

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Acute hemorrhagic conjunctivitis
Acute hemorrhagic cystic
AIDS/acquired immune deficiency syndrome—human immunodeficiency virus
Bronchiolitis—respiratory syncytial virus
California encephalitis—California encephalitis virus
Cervical cancer—human papilloma virus
Chickenpox—varicella zoster virus
Colorado tick fever—Colorado tick fever virus
Conjunctivitis—herpes simplex virus
Cowpox—vaccinia virus
Group, infections—parainfluenza viruses
Dengue—dengue virus
“Devil’s Grip”—coxsackie B
Eastern equine encephalitis—EEE virus
Ebola hemorrhagic fever—Ebola virus
Gastroenteritis—Norwalk virus
Genital HSV—herpes simplex virus
Gingivostomatitis—HSV-1
Hantavirus hemorrhagic fever/Hantaan-Korean hemorrhagic fever—Hantavirus
Hepatitis
Hepatitis A—hepatitis A virus
Hepatitis B—hepatitis B virus
Hepatitis C—hepatitis C virus
Hepatitis D—hepatitis D virus
Hepatitis E—hepatitis E virus
Herpangina—coxsackie A
Herpes, genital—HSV-2
Herpes labialis—HSV-1
Infectious myocarditis—coxsackie B1–B5
Infectious pericarditis—coxsackie B1–B5
Influenza—influenza viruses A, B, and C
Keratoconjunctivitis—adenovirus
Lass hemorrhagic fever—Marburg virus
Measles—rubella virus
Meningitis, aseptic—coxsackie A and B (enterovirus), lymphocytic choriomeningitis virus
Mononucleosis—Epstein–Barr virus
Mumps—mumps virus
Pharyngitis
Respiratory syncytial virus
Influenza virus
Parainfluenza virus
Adenovirus
Epstein–Barr virus
Pleurodynia—coxsackie B
Pneumonia, viral—respiratory syncytial virus
Polio, poliomyelitis—Poliovirus
Progressive multifocal leukoencephalopathy—JC virus
Rabies—rabies virus
Roseola—HHV-6
Rubella—rubivirus
Severe acute respiratory syndrome (SARS)—a human coronavirus
Shingles (zoster)—varicella zoster virus
Urethritis—herpes simplex virus
West equine encephalitis—WEE virus
Yellow fever—Yellow fever virus
Zoster—varicella zoster virus

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## ASSESSMENT OF CURRENT AND POTENTIAL ANIMAL MODELS IN VIROLOGY

A number of potential useful models exist that may facilitate and improve the understanding of the pathogenesis and treatment of viral infection. A good example is HIV-1 disease.

Animal models have provided a controlled setting for the study of human immunodeficiency virus-1 (HIV-1) disease, the preclinical testing of novel antiviral compounds, and the evaluation of vaccines. Because the animals serve as models for humans they should be closely reflective of human physiology and pathophysiology. Moreover, their use must be complementary to, and not replaceable by, experimental approaches that do not require animals. For any given experiment, the critical question is which, if any, model is most useful—and why?

## ADVANCES MADE IN BIOMEDICAL RESEARCH IN VIROLOGY USING ANIMALS

Viruses are collection of genetic information directed toward replication. The viral genome contains the blueprint for virus replication encoded in the genetic code and must be decoded by molecular biology. Exploitation of viral diseases of animals may ultimately provide a useful means of understanding human viral diseases.

The literature on the history of the use of animals in human medical virology is somewhat sketchy and not well organized. This clearly indicates that the study of viruses as a pathogen in animal models is a relative new field and in most cases, due to molecular biology and genomic research studies, is now coming into its own.

Most of the major advances in modern virology during the past 25 years have been due principally to the development of refined laboratory techniques and tools and have provided an array of new knowledge and information about the nature of viral infection and pathogenesis.

Changing their role from hunters of microbes to biochemists probing the nature of life, virologists are simultaneously reflecting and leading the revolution in biomedical research. By using the post-World War II tools of tissue culture, radioactive isotopes, chromatography, density gradient centrifugation, and the electron microscope, they have acquired vast knowledge about the way viruses infect cells and cause disease. Unexpectedly, the viruses themselves have emerged as powerful probes into the nature of cellular and life processes. Because of the necessarily close relationship between viruses and their host cells, the understanding and control of viral infections depend almost wholly on knowledge of the biochemistry of cells.

Vaccines and sera have been powerful aids in the prevention of viral diseases such as polio, measles, mumps, rubella, yellow fever, and hepatitis, but they are only extensions of the fundamental principles of Jenner's smallpox vaccine and Pasteur's rabies vaccine. Compared with the modern treatment of established diseases by means of specific chemotherapy, the management of viral diseases lags behind all other forms of chemotherapy.

## ACHIEVEMENTS OF ANIMAL RESEARCH IN VIROLOGY

Several animal models, primarily rodents and monkeys, have been developed that recapitulate many aspects of the disease seen in humans. Studies reviewed here demonstrate how these models

have played a vital role in understanding and developing therapeutics for these viral diseases.

**HUMAN PAPILLOMAVIRUS AND CERVICAL CANCER** As the scientific community continues to make new discoveries about factors contributing to the development of cancer, viral pathogens have been found to play an important role. In the development of cervical cancer, one specific type of abnormal cell growth (neoplasia), DNA from human papillomavirus (HPV), has been detected in 50–95% of lesions. It is estimated that women with HPV have 10–30 times the risk of developing cervical neoplasia than those not infected. Using this system as a model, many questions on how viral infections influence the development of cancer have been explored experimentally.<sup>26</sup>

**INFLUENZA VIRUS** The animal models presently available for the study of the pathogenesis of influenza virus disease have limitations.<sup>27,28</sup> Influenza A virus will experimentally infect a number of Old World and New World primates. The gibbon and baboon develop clinical illness with nasal application of the virus, and the squirrel, cynomolgus, and rhesus monkeys develop illness when the virus is inoculated intratracheally. Primate models suffer from a number of disadvantages, including the limited availability of expensive animals. In addition, these animals are outbred and the models lack many of the reagents necessary to characterize the host response in detail. Mammals such as horses and pigs that are natural hosts for influenza have also been used experimentally. However, their large size and the limited number of reagents available preclude their use in the laboratory.

Small-animal models that have been used to study influenza virus pathogenesis include the ferret, in which human influenza virus was originally isolated.<sup>29</sup> Adult ferrets become ill after infection with unadapted influenza A viruses, exhibiting fever, lethargy, and weight loss. The ferret model has been used in recent studies of H5N1 viruses, the transmission of influenza, and the development of resistance to antiviral therapy. Unfortunately, ferrets are outbred and reagents are not available for dissecting the correlates of protective immunity.

There has been one report, published in Polish and largely overlooked, of the use of outbred cotton rats (*Sigmodon hispidus*) for pathogenesis experiments with influenza viruses. Nasal administration of virus in lightly anesthetized cotton rats resulted in virus replication, the production of pulmonary lesions, and a strong immune response. Results suggest that the cotton rat may serve as a useful model for the study of influenza pathogenesis. The animals are small, inbred, easy to handle, and relatively inexpensive to purchase and maintain.<sup>30</sup>

Influenza virus strains that cause worldwide outbreaks (pandemics) are classic examples of emerging viruses that are maintained in other animal hosts before transmission to humans. Influenza viruses are isolated from a variety of animals, including humans, pigs, horses, wild and domestic birds, and even sea mammals.<sup>31</sup> The most devastating viral infection in this century was not caused by HIV, but by Spanish influenza, which killed more than 20 million people worldwide. Genetic studies suggest that the Spanish influenza virus originally was derived from birds. Furthermore, the causative viruses from the 1957 and 1968 influenza pandemics were hybrids between human and avian influenza viruses. Because humans did not have immunity to avian influenza viruses, the hybrid viruses produced devastating consequences (70,000 and 46,500 deaths globally in the 1957 and 1968 pandemics, respectively). Thus, it is critical to understand the

mechanisms by which new influenza strains capable of causing pandemics emerge. Animal models will play a critical role in understanding the mechanisms.

**HANTAVIRUS** Hantavirus is segmented RNA viruses belonging to the genus *Hantavirus* in the family Bunyaviridae. Hantaviruses are maintained in various rodent reservoirs, in which the hosts are persistently infected without disease symptoms. Specific hantaviruses transmitted from the contaminated urine and feces of infected rodents cause two important human diseases, hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS). Annually, hundreds of thousands of cases of HFRS are reported throughout Euro-Asia, whereas hundreds of cases of HPS are reported in countries in North and South America. Because rodents act as the natural reservoir for hantaviruses and human-to-human infections are rare, understanding the ecology of hantavirus within their natural reservoir is important for preventing and controlling the emergence of such diseases.<sup>32</sup>

The comparison of many hantavirus genomes from different rodent species has shown a clear correlation between the rodent species and the virus genotype, suggesting that hantaviruses have coevolved with their natural hosts for >20 million years, since before the first humans evolved. It remains unclear how hantaviruses exist within a rodent reservoir, particularly how they establish a persistent infection. In experiments with laboratory rats and mice, several groups have shown that an experimentally infected newborn animal readily develops a persistent infection, whereas an adult animal develops only a transient infection and recovers completely. On the other hand, epizootiological investigations have demonstrated that virus is transmitted between adult animals through wounds, and the adults develop a persistent infection.

**EBOLA VIRUS** Ebola virus is a nonsegmented RNA virus, which, together with Marburg virus, makes up the filovirus family. This now notorious group of viruses was discovered in 1967 when Marburg virus was identified as the etiological agent of a hemorrhagic fever outbreak in research facilities in Europe, which handled tissues from African green monkeys imported from Uganda. Subsequently, Ebola viruses were shown to be the cause of simultaneously occurring hemorrhagic fever outbreaks in 1976 in the Democratic Republic of Congo (DRC, formerly Zaire) and Sudan. These outbreaks were shown to be caused by two different subtypes of Ebola virus, which became known as the Zaire and Sudan subtypes. Mortality rates of up to 80% were recorded in these and more recent outbreaks in DRC and Gabon in 1995–1996. Epidemiological data from recent outbreaks indicate that close contact is necessary for efficient transmission of Ebola virus from one individual to another, and little evidence can be found for aerosol transmission of the virus. Despite considerable efforts to identify the natural reservoir for Ebola and Marburg viruses, the host species remains an enigma. Although nonhuman primates have been implicated as the source of the introduction of the virus into humans during several of the identified outbreaks, they are not considered likely to represent reservoir species because of their susceptibility to high-mortality hemorrhagic disease similar to that seen in humans.

**NIPAH VIRUS** Nipah virus is a newly discovered member of the paramyxovirus family of nonsegmented RNA viruses. This virus was responsible for a viral encephalitis outbreak in Malaysia that was first recognized in October 1998 and ended in midsummer

1999. This outbreak resulted in almost 300 confirmed infections, and the mortality rate for hospitalized cases was approximately 35%. Initially, Malaysian authorities thought the outbreak was caused by Japanese encephalitis (JE virus, a mosquito-borne RNA virus). However, JE vaccination and mosquito control efforts failed to halt the epidemic. The virus appeared to be first introduced into pigs, where close contact caused by intensive farming practices led to efficient pig-to-pig transmission, and subsequently pig-to-human transmission. Most human cases were in close proximity to the infected pigs. Genetic analysis showed Nipah virus to be closely related to Hendra virus, which recently was discovered in Australia as a cause of disease in horses and humans and also is maintained in *Pteropus* species fruit bats.

These examples highlight the subtle balance of environmental and genetic factors that can mold the diverse evolutionary patterns observed for RNA viruses and illustrate the complexity of these systems, which makes it difficult to predict future viral disease emergences. Much more research is needed and animal models will continue to be needed to ultimately understand and control these diseases.

## RELEVANT ANIMAL MODELS

Use of animals as models for human disease has been indispensable in understanding the cause, biology, and prevention of disease. The Animal Model Division at the Institute of Human Virology has developed several relevant animal models, particularly for the study of AIDS and AIDS-associated cancers.

Animal models are required for the study of human diseases. However, the relevance of small animal models such as mice to natural human *in vivo* physiological and metabolic kinetics remains unclear. Large animal species may provide far more appropriate preclinical models that will more closely reflect human physiological characteristics and behavior. Among large animals, nonhuman primates may provide the best models because of their close phylogenetic relationship to humans. It is essential to develop animal models for human diseases to reveal its mechanisms and to develop new therapeutic interventions.

Led by the author of this review at the Institute of Human Virology, the Animal Models Division is a unique feature of the Institute, enabling scientists to work with relatively inexpensive models to study AIDS and new drugs or therapies without risk to humans. Developing animal subjects for use in viral research is a science unto itself, and it is essential in taking a discovery from laboratory to clinic. The use of animals as models for human disease has been indispensable in understanding the causes, biology, and prevention of disease. “To my knowledge, almost all major scientific successes on unraveling and conquering human diseases have been with the use of animal models. AIDS and AIDS-associated diseases, will be no exception” (J.L. Bryant, personal communication).

## VIRAL DISEASE IN SEARCH OF AN ANIMAL MODEL

Hepatitis viruses belong to different families and have in common a striking hepatotropism and restrictions of propagation in cell culture. Viral hepatitis represents a global public health problem. Over the past 20+ years, the chimpanzee model has served as the backbone for advancements in the hepatitis C virus (HCV) research field. Despite this remarkable progress, the chimpanzee model has some important disadvantages. Perhaps most

importantly, chimpanzees are rare, expensive, and difficult to handle. Limitations to the chimpanzee model have stimulated progress toward developing alternative animal models for HCV research. Transgenic technology, coupled with the relative ease and low cost by which mice can be reared and maintained, along with the availability of inbred mouse strains, have made the laboratory mouse an attractive animal model for HCV research.

For some human viruses [e.g., hepatitis B (HBV) and HCV], no satisfactory cell culture or animal model exists, and in these cases, inhibition of an essential viral function or activity against related viruses can be used to indicate potential activity. When no satisfactory cell culture or animal model exists for the target human virus, it is particularly important to know whether a drug's active moiety enters cells, if it has a proposed intracellular site of action, and if the intracellular concentration is consistent with biochemical studies to identify an inhibitory concentration. Cell-based assays and host cell lines for studying viruses such as HBV and HCV replication may advance and improve, but at the present time are limited. For analysis of HCV replication, a replicon system has been developed that permits studies of viral replication and can be used to assess antiviral activity of some anti-HCV drugs.

More than 200 million people worldwide are now believed to be infected with HCV, including 4 million individuals within the United States. HCV accounts for the deaths of at least 8000 to 10,000 Americans each year and is now one of the most common indicators for adult liver transplants in developed countries. Before the introduction of anti-HCV screening in mid-1990, HCV accounted for 80–90% of new cases of posttransfusion hepatitis in the United States. Currently, injection drug use is probably the most common risk factor for HCV infection, with approximately 80% of this population seropositive for HCV. A high rate of HCV infection is also seen in individuals with bleeding disorders or chronic renal failure, groups that have had frequent exposure to blood and blood products.

HCV is a global public health problem, with approximately 3% of the world population now infected. The clinical course of HCV often involves chronic infection, which can lead to liver dysfunction and hepatocellular carcinoma. Because HCV cannot be efficiently propagated in cell culture, research has relied heavily on animal models to study the physical characteristics of HCV and the course of events associated with HCV infection. The chimpanzee is the only nonhuman primate actually proven to be susceptible to HCV infection and has commonly been used to study viral hepatitis induced by HCV. Molecular cloning of the HCV genome has now allowed HCV transmission studies in chimpanzees to progress from the early work of characterizing infectious serum to a current focus of characterizing infectious HCV molecular clones. Moreover, the cloned HCV genome has paved the way for the development of alternative animal models for HCV, most notably transgenic mouse models for the study of HCV pathogenesis.<sup>33</sup> The expression of specific viral protein products in these animal models will provide important insights into the structure–function relation that specific HCV genome sequences impart on virus replication and pathogenesis.

Despite this remarkable progress, the chimpanzee model has some important disadvantages. Perhaps most importantly, chimpanzees are rare, expensive, and difficult to handle, and must be housed and cared for in appropriate nonhuman primate research facilities. Such research facilities must possess proper surgical support and specialized veterinary care. Moreover, the chimpan-

zee has been listed as an endangered species since 1988, and appropriate safeguards must be considered whenever selecting such a model for research.

These and other limitations to the chimpanzee model have stimulated progress toward developing alternative animal models for HCV research. Transgenic technology, coupled with the relative ease and low cost by which mice can be reared and maintained, along with the availability of inbred mouse strains, have made the laboratory mouse an attractive animal model for HCV research.<sup>34</sup> An HCV infection cannot be propagated in mouse tissues, which obviously limits the research application of mouse models. However, expression of the HCV genome or subgenomic fragments of HCV within inbred strains of mice has demonstrated the utility of mouse models for research geared toward understanding mechanisms of HCV pathogenesis.

Transgenic mice expressing HCV proteins are only beginning to provide insights into the pathobiology of HCV infection. From the studies presented, it is clear that expression of HCV proteins per se is not directly cytopathic to hepatocytes, even when expression levels are greater than those observed in HCV-infected individuals. This evidence supports the notion that the host immune response to HCV may play a significant role in HCV pathogenesis. The use of conditional transgenic expression systems, such as the Cre/LoxP system, offers exciting prospects to study the immune-mediated mechanisms of HCV-related liver injury in transgenic mice. However, the recent development of mice immunocompetent for the HCV proteins using this system awaits full characterization. It is clear that in some transgenic mouse strains, expression of the HCV genome or the core protein alone results in the development of steatosis and HCC. How does the core protein induce these pathologies, and how may the other HCV proteins contribute to this phenotype? It is unclear whether this phenotype is a result of HCV protein expression directly or whether it occurs in combination with environmental or host-derived factors leading to chronic liver cell injury. Importantly, steatosis and HCC are relevant clinical manifestations seen in HCV-infected individuals. These transgenic mice should therefore play a pivotal role in elucidating the molecular mechanisms of HCV-related liver pathology.

## CONCLUSIONS

There should be no doubt that the knowledge gained from the study of viral diseases in humans in different animal species has enhanced our knowledge and understanding of the pathogenesis of the diseases in humans. One important component of the value of using animals in viral research is that the pooling of knowledge of diseases in different animal species has led to more rapid progress in understanding the pathogenesis of diseases.

It was not the intent here to discuss comprehensively the contribution of animals in viral research but to historically acknowledge that they have played and will continue to play a major role in solving many health problems, including viral diseases in humans. A review of the literature clearly shows that depending on the training and background of the scientists concerning the use of animals in viral research, there are numerous differences of animal models in viral research. It is clear that the more knowledge the scientist has of diseases in animals the better the understanding of how a model can be used. For example, the direct application of knowledge of a disease in an animal, scrapie in sheep, has led to a better understanding of a human disease,

kuru. It was a veterinarian and pathologist, Dr. W. J. Hadlow, who through his experience with scrapie in sheep and goats noted the similarities of the histological lesions in the brains of kuru patients and those of sheep and goats. He suggested the inoculation of brain material from kuru patients into animals. Dr. Carlton Gajdusek, using chimpanzees, produced a fatal disease in these animals after a long inoculation period. This established a viral etiology for kuru and led to the prevention of the diseases. This is one of numerous examples of how applying the knowledge of veterinary medicine and human medicine can lead to the prevention and treatment of diseases caused by viruses.

Our understanding of emerging and reemerging viruses from an evolutionary perspective offers clear directions to follow. If we seriously plan to develop efficient and reasonable control strategies, an international concerted effort involving a thorough survey of the genetic diversity of viruses in time and space is a must. This will also help in understanding several processes that take place at the molecular level in a comparative context. More emphasis on multidisciplinary sciences is needed. Molecular biology and systematics, bioinformatics, data mining, and analysis are all becoming considerably sophisticated lately. Hepatitis C and HIV-1 are glaring proof that disease knows no national borders. The use of and development of animal models for studying viral diseases of humans will continue to be a necessary part of biomedical research in understanding the current viral diseases that affect humans and the new emerging viruses.

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