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Of mice, rats and men: small animal model of hepatitis C virus infection

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Alexander Graham Bell has been quoted as saying, “When one door closes, another opens; but we often look so long and so regretfully upon the closed door that we do not see the one which has opened for us.” This quotation is particularly apt when considering animal models of hepatitis C virus (HCV) infection. For over 40 years *Pan troglodytes* (common chimpanzees) served as an exceptionally valuable animal model for HCV research. As the only species with known susceptibility to infection, they were essential for discovery of HCV as the cause of non-A, non-B hepatitis, parsing mechanisms of protective immunity, vaccine development, and research leading to direct acting antivirals (DAA) that profoundly changed treatment of chronic hepatitis C. That door closed in 2013 when the NIH placed new restrictions on chimpanzee use in biomedical research. Efforts to develop a more tractable animal model met with partial success. For instance, expression of human CD81 and occludin in the liver of laboratory mice facilitated HCV entry, but replication also required ablation of innate immune signaling pathways [1]. Barriers to spontaneous entry and replication of HCV have therefore limited studies of pathogenesis and vaccine development in laboratory mice.

New doors in HCV animal modeling may now be opening. Two recent publications described experimental infection of mice [2] and rats [3] with a hepacivirus isolated from feral Norway rats (*Rattus norvegicus*) trapped in New York City. These small animal models will undoubtedly help to establish further understanding of the pathogenesis of hepaciviruses. Like HCV, the rat virus is classified within the Hepacivirus genus of the

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Flavivirus family. Key characteristics it shares with HCV include genome organization, polyprotein cleavage sites, conserved 5' and 3' UTR secondary structure, and functional microRNA 122 binding sites in the 5' UTR critical for liver tropism [3]. The mice and rats were immunocompetent and replication did not require expression of human entry factors in hepatocytes. It will be of interest to assess whether these rodent hepaciviruses also utilize important cellular receptors that have thus far been identified for canonical HCV entry. Mice challenged with the rat hepacivirus spontaneously resolved infection within 5-7 weeks. This was temporally associated with adaptive humoral and cellular immune responses considered important in control of human HCV infection. With regard to cellular immunity, transient antibody-mediated depletion of CD4+ T helper cells from mice before challenge with the rat hepacivirus resulted in chronic infection characterized by apparent exhaustion of CD8+ cytotoxic T cells that expressed high levels of the inhibitory molecule programmed cell death 1 (PD-1). This observation is consistent with an essential role for CD4+ T cell immunity in control of human primary HCV infections, where spontaneous resolution is associated with a sustained helper cell response. It is also notable that immune mice developed a secondary infection when rechallenged with rat hepacivirus 4 to 7 months after spontaneous resolution of acute primary infection. Secondary infection occurred despite the presence of low-titer serum neutralizing antibodies. An accelerated virus-specific CD8+ T cell response was kinetically linked with rapid clearance of the second infection. These findings also align well with observations from HCV-infected humans and chimpanzees, where resolution of primary infection sharply reduces the risk of persistence upon re-exposure to the virus [4]. There is direct evidence that this immunity is mediated by CD4+ [5] and CD8+ T cells [6], and perhaps neutralizing antibodies targeting the HCV recombinant envelope glycoproteins. This mouse model may be particularly well suited to investigate the importance of the humoral immune response in clearance of secondary hepacivirus infections, a hypothesis supported by some human studies [6].

Hepacivirus infection outcome was different in rats. Unlike mice, infection persisted in almost all inbred and outbred rats challenged with the virus. Persistent replication in liver was associated with formation of lymphoid aggregates, parenchymal inflammation, as well as macro and micro steatosis that is observed in chronic hepatitis C of humans. Persistent activation of type I interferon signaling pathways, also a common feature of chronic hepatitis C in humans, was observed. There is as yet no information on immunity or why it fails to prevent chronic infection in most rats. With further characterization, the rat model may be of particular importance in evaluating vaccines for protection from hepacivirus persistence. It is also notable that persistent replication of the rat hepacivirus was suppressed or cured with Sofosbuvir, a DAA targeting the HCV NS5b polymerase protein. Thus, the rat model may be ideally suited to determine if adaptive immunity recovers after antiviral cure of chronic hepacivirus infection, or provides protection from rechallenge with the virus.

Rodent hepacivirus models offer obvious advantages. They include access to liver where the virus replicates, infection of genetically identical or modified rodents, and use of clonal hepaciviruses that can be mutated to identify structural features important for replication and immune evasion. Impaired infection in rats challenged with clonal hepaciviruses carrying mutations in the 5' UTR miR-122 seed sites demonstrates this principle [3]. These models will almost certainly have some limitations, but they are difficult to predict given the

preliminary characterization of infection and immunity. As highlighted above, some mechanisms of protective immunity and chronic liver injury may overlap in rodents and humans. However, it must also be acknowledged that the hepaciviruses and their rodent or human hosts are sufficiently different that extrapolation of findings should be undertaken with caution. Failure of the rat hepacivirus to spontaneously establish persistent infection in mice could complicate assessment of vaccines designed to protect against persistence, a key goal of human vaccination. Moreover, how HCV subverts the human CD4⁺ T cell response to establish persistent infection remains unknown. Comparison of successful and failed helper responses is not yet possible in mice or rats because of partial or complete skewing of infection outcome towards resolution or persistence, respectively. Persistence in rats but not mice may reflect a high level of rat virus adaptation for its natural host. Establishing a balance between resolution or persistence of hepacivirus infection in these species may be accomplished by experimental adaptation of virus or the murine and rat hosts. Understanding the difference in infection outcome between these species may be particularly important in unlocking the mystery of acute resolving and persisting infections in humans.

The need for a new model of HCV infection is acute. DAA therapy will likely have limited impact on HCV disease burden in the foreseeable future. Most HCV infections are undiagnosed and virus transmission is increasing in many regions of the world because of an epidemic in opioid use. There is no vaccine to prevent HCV transmission. Hopes are now pinned on a single vaccine approach that elicits T cell immunity against non-structural HCV proteins. Proof of concept studies in chimpanzees suggest that this unique approach might prevent HCV persistence [7]. Immunization of human volunteers with recombinant virus vectors expressing these non-structural HCV proteins also generated pan-genotypic T cell immunity. The vaccine is now being evaluated for protection in individuals at risk for infection [8].

Despite this progress, mechanisms of protective immunity remain uncertain. Whether T cells, antibodies, or both will be required to protect against primary HCV infection, or reinfection after costly antiviral cure of chronic hepatitis C, is unknown. The door to chimpanzee HCV research is now firmly closed, but a timely new door has opened with the discovery of a highly related hepacivirus that infects rats and mice. It represents a critically important advance with the potential to provide insight into subversion of immunity by hepaciviruses and facilitate further development of vaccines to stop transmission of virus that continues to impose a significant public health cost.

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