

Within host RNA virus persistence: mechanisms and consequences[☆]

Richard E Randall¹ and Diane E Griffin²



In a prototypical response to an acute viral infection it would be expected that the adaptive immune response would eliminate all virally infected cells within a few weeks of infection. However many (non-retrovirus) RNA viruses can establish 'within host' persistent infections that occasionally lead to chronic or reactivated disease. Despite the importance of 'within host' persistent RNA virus infections, much has still to be learnt about the molecular mechanisms by which RNA viruses establish persistent infections, why innate and adaptive immune responses fail to rapidly clear these infections, and the epidemiological and potential disease consequences of such infections.

Addresses

¹ School of Biology, University of St. Andrews, Scotland, UK

² W. Harry Feinstone Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA

Corresponding author: Randall, Richard E (rer@st-andrews.ac.uk)

Current Opinion in Virology 2017, 23:35–42

This review comes from a themed issue on **Viral pathogenesis**

Edited by **Michael Diamond**

<http://dx.doi.org/10.1016/j.coviro.2017.03.001>

1879-6257/© 2017 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

Infections with most non-retroviral RNA viruses cause characteristic signs and symptoms of an acute disease. During the acute phase of infection, the virus replicates rapidly and is shed into the environment with spread to new susceptible individuals. Recovery is typically associated with virus clearance and establishment of varying lengths of immunity to re-infection. For such viruses to be maintained within a population there needs to be a continuous supply of susceptible individuals to sustain the transmission cycle. For viruses, other than endogenous retroviruses and viruses transmitted vertically, if the host population size and/or density is small, as was likely the case during most of human evolution and is currently

the situation for many animal populations, the number of susceptible individuals may not remain high enough for the virus to maintain itself within a host population. Conversely, if the population density is very high, for example in bat colonies, virus spread may be extremely rapid thereby also leading to a decrease in the numbers of susceptibles (through the induction of long-lasting protective immunity [1] and/or through high mortality rates) to levels below that required for continued virus transmission [2]

Because viruses are obligate intracellular parasites that must be maintained in a population, RNA viruses have evolved a number of strategies to counteract the potential problem of 'running out' of susceptible individuals, such as: (i) a high mutation rate that results in ongoing immune selection of antigenic variants (*e.g.* influenza virus), (ii) infection of mucosal surfaces, where it is difficult to induce long lasting protective immunity, resulting in repeated infections with the same virus (*e.g.* respiratory syncytial virus) or (iii) infection of multiple species, thereby increasing the numbers of susceptible individuals. As an additional strategy some viruses, such as hepatitis C virus (HCV) and Borna disease virus (BDV), have evolved specific mechanisms that lead to the establishment of persistent infections in at least some individuals who can then act as reservoirs for the virus within a host population.

In a prototypical response to an acute viral infection it is expected that a virus will be cleared by innate and adaptive immune responses within a few weeks of infection. Therefore, any infection that persists longer than this may be considered persistent, even if it does not lead to life-long infection with the production of infectious virus. Indeed, for foot and mouth disease virus (FMDV), cattle are considered persistently infected if infectious virus can be detected for more than 28 days after infection [3,4]. Not all persistent infections may represent a selective advantage for a virus. Thus viruses, or viruses with defective genomes, may persist and slowly spread from cell to cell without the production of infectious virus. Such infections may nevertheless have important consequences for the host in that they may lead to chronic or even fatal disease outcomes, for example persistent measles virus (MeV) infections of the brain can lead to subacute sclerosing panencephalitis (SSPE) [5,6], or they may induce life-long immunity thereby preventing reoccurrence of the acute infection, as has been suggested for MeV [7]. It is this broad definition of persistence that we

[☆] This opinion piece takes its title from a conference with the same name that was held in St Andrews, Scotland on 24th–26th August 2016

will use in this opinion piece as we consider some of the outstanding issues regarding ‘Within host RNA virus persistence’.

Epidemiological consequences of persistent infections

The ability of some RNA viruses, for example HCV and BDV, to establish persistent infections in a significant proportion of infected hosts is critical for them to be maintained within their host communities. However, the importance of persistent infections for the epidemiology of other viruses has yet to be established (Table 1), particularly those that cause acute infections with obvious clinical outcomes. However, the example of FMDV is instructive. Although FMDV is best known for causing devastating outbreaks of acute disease in domesticated ungulates (*e.g.* cows and sheep), it can also establish persistent infections in some animals (persistently infected sheep and goats can shed virus for up to 9 months, cattle for up to 2.5 years and buffalo for >5 years) which can act as a source of FMDV in future outbreaks [3,4].

Similarly, swine vesicular disease virus may be able to establish persistent infections in pigs (>100 days) that may act as carriers of swine vesicular disease [8]. There is also evidence that acute respiratory viruses, such as rhinoviruses [9,10] and respiratory paramyxoviruses [11], establish persistent infections in some individuals with production of infectious virus for many weeks or months, although such infections are often, but not always, associated with immune dysfunction (see below) and/or age. Also, whilst many arboviruses are able to establish inapparent life-long persistence in their arthropod vector [12], they often cause significant acute disease in their vertebrate hosts, although their ability to establish persistence in vertebrates may have been underestimated [13].

Even such obviously acute virus infections such as Zika virus and Ebola virus in humans can persist in very small numbers of individuals over a period of months, and perhaps years, and such persistently infected individuals

Table 1

Examples of viruses that can establish persistent infections, including in immunocompromised hosts, and some associated references

Arenaviridae, Mammarenaviruses, for example lymphocytic choriomeningitis virus [81]
Arteriviruses, for example equine arteritis virus, simian haemorrhagic fever virus [82]
Bornaviridae, for example Borna disease virus [83]
Bunyaviridae Hantaviruses [37,38,84]
Caliciviridae Noroviruses, for example Norwalk virus [85]
Coronaviridae, for example mouse hepatitis virus [86]
Endornaviridae and Partiviridae; [87]
Filoviridae, Ebolaviruses, for example Ebola virus [20,68] Marburg viruses, for example Marburg virus [88]
Flaviviridae Hepaciviruses, for example hepatitis C virus [89] Pegivirus, for example GB viruses [90] Flavivirus, for example West Nile virus [91], Zika [15,67], Japanese encephalitis virus [92], tick borne encephalitis virus [93] Pestiviruses, for example Bovine viral diarrhoea virus [70,94,95]
Nodaviridae, for example flock house virus of insects [35,96]
Orthomyxoviridae, for example influenza viruses [97,98]
Paramyxoviridae [11] Morbilliviruses, for example measles virus [99], canine distemper virus [100], feline morbillivirus [101,102] Respiroviruses, for example parainfluenza virus type 3 [103] Rubulaviruses, for example parainfluenza virus type 5 [104], porcine rubulavirus [105]
Picornaviridae Aphthoviruses, for example foot-and-mouth disease virus [4] Cardioviruses, for example Theilers murine encephalomyelitis virus [106] Enteroviruses, for example poliovirus [62], coxsackievirus [31], rhinoviruses [9,10], swine vesicular disease virus [8]
Pneumoviridae, for example respiratory syncytial virus [107–109]
Reoviridae Orbivirus, for example bluetongue virus [21–23] Phytoreovirus, for example rice gall dwarf virus [34]
Rhabdoviridae Lyssaviruses, for example rabies virus [110,111]
Togaviridae Alphaviruses, for example Chikungunya virus [112] Rubiviruses, for example rubella virus [113]
See also arboviruses [13]

can occasionally transmit the virus, thereby being a potential source of future outbreaks [14,15]. Whilst no one would suggest that the ability of Ebola virus to establish persistent infections has evolved in humans, the fact that even small numbers of individuals can become persistently infected may reflect its intrinsic ability to establish persistence in its natural host.

One potential advantage of persistent infection for the infected host may be the maturation of the antiviral immune response and development of long-lasting protective immunity. For instance, persistence of measles virus RNA and protein in lymphoid tissue for months after the primary infection stimulates continued production of plasmablasts producing MeV-specific antibody of increasing avidity, T cells of evolving functionality and the establishment of life-long immunity that characterizes recovery from measles [16,17]. Thus, there may be clear advantages both for the virus and host in evolving towards the establishment of persistent infections, especially because virus: host interactions that result in persistent infections are unlikely, in most cases, to lead to high levels of mortality.

Disease consequences of persistence

Although most persistent RNA virus infections are probably inapparent, persistent infections can sometimes lead to chronic disease or relapses of acute disease. Long-recognized examples include hepatocellular carcinoma and liver fibrosis as a late consequence of HCV infection [18], and SSPE following measles virus infection. Indeed, the CNS, as an immunologically privileged site (see below), is an organ in which RNA viruses can often establish persistent infections with disease consequences [5,19]. Recent examples include reactivation of CNS infection after apparent recovery from Ebola virus disease [14,20]. Other chronic human diseases have also been linked to persistent RNA virus infections, some controversially, including Paget's bone disease, multiple sclerosis, otosclerosis, post-polio syndrome and other late neurodegenerative diseases, chronic fatigue syndrome, certain autoimmune diseases and exacerbation of chronic obstructive pulmonary disease (Table 2).

The mechanisms by which persistent RNA virus infections induce chronic disease is poorly understood, but it has been suggested that chronic stimulation of inflammatory responses may be an important driving factor (see M. K. McCarthy and T. E. Morrison, Persistent RNA virus infections: do PAMPs drive chronic disease, in this issue of Current Opinions in Virology).

Mechanisms of persistence

To establish persistent infections viruses must (i) avoid elimination by the host's immune response, and (ii) avoid killing all infected cells whilst maintaining their genomes within some infected cells. This may entail low level virus

Table 2

Examples of human diseases associated, sometimes controversially, with persistent RNA virus infections and some associated references

Autoimmune diseases: various viruses [114,115]
Chronic fatigue syndrome: enteroviruses [116]
Exacerbation of chronic obstructive pulmonary disease: Respiratory syncytial virus [108]
Liver cirrhosis/cancer; hepatitis C virus [117]
Multiple sclerosis: a number of RNA and DNA viruses [118,119]
Paget's bone disease: Measles and other paramyxoviruses [11,120]
Persistent arthralgia: Chikungunya virus [121]
Post-polio syndrome: poliovirus [122]
Progressive rubella panencephalitis; Rubella [113]
Subacute sclerosis panencephalitis and measles inclusion encephalitis: measles virus [123]
Olfactory dysfunction; parainfluenza virus type 3 [124]
Otosclerosis: measles virus [125,126]

replication within cells that themselves remain persistently infected (*e.g.* BDV), infections in which the virus slowly spreads from cell to cell, but during which the infected cells may die (*e.g.* rabies virus), or infections in which the virus passively hides without apparent replication (*e.g.* BTV in erythrocytes [21–23]). Both viral and host factors will influence the type of persistent infection established. For viruses that clearly need to establish persistent infections to survive in nature the molecular basis by which they do so must be an evolved process. However, this is unlikely to be the case for viruses where the establishment of persistence has no obvious selective advantage for the virus, unless the general way the virus replicates reflects the need of an ancestral virus to establish persistent infections, or the virus establishes advantageous persistent infections in a different host.

Repression of virus transcription and replication

If a cell continues to synthesize high levels of viral proteins, the cell will probably die either as a direct consequence of virus replication or be eliminated by innate and adaptive immune responses. Therefore, to establish persistent infections virus replication probably needs to be repressed in at least some infected cells. Long-lived cells such as cardiac myocytes and brain and spinal cord neurons may be particularly likely to restrict virus replication and avoid immune-mediated elimination (see below). However, RNA viruses can establish persistent infections in a variety of tissues, not all of which are immune privileged sites. How repression of virus replication is achieved in these cells, even for a well-studied virus such as HCV, is usually not clear. HCV appears to have evolved mechanisms to hijack cellular factors, such as microRNAs that bind its genome to protect it from degradation by the 5'–3' exoribonuclease Xrn1 [24], inhibit its detection by sensors of the innate immune response and to regulate virus transcription, replication and genomic RNA abundance [25]. BDV, linked to

neurobehavioral disorders, is the only member of the order Mononegavirales that replicates in the nucleus and non-cytolytically infects animals to establish persistence [26]. To maintain persistence, BDV tethers its genome to host cell chromosomes, so that both daughter cells remain infected when the cell divides [27]. BDV also blocks apoptosis, and thus promotes persistence by preventing cell death, through the action of its accessory protein X [28], which may influence the establishment and reactivation of BDV through its regulatory function on virus polymerase activity [29]. Furthermore, 5'-terminal trimming, a mechanism that leads to the loss of terminal nucleotides from the BDV genome, may attenuate virus replication and transcription (thereby also helping to facilitate virus persistence), and prevent the genome from activating innate immune responses, which also strongly influence the outcome of virus infections (see below) [30]. Similar modifications (deletions and insertions) at the 5' and/or 3' ends of the genomes of other RNA viruses, including LCMV, hantaviruses and coxsackieviruses [31–33], may also play similar roles in influencing their ability to establish persistent infections. Certain insect and plant viruses also appear to have evolved mechanisms, dependent upon the insect siRNA defense system, to dampen down their replication thereby facilitating virus persistence. Here, viral dsRNA produced during virus replication is recognized by Dicer-2 (DCR2), a central component of the siRNA pathway, that processes the RNA to produce virus-derived siRNAs. These are subsequently recognized by the RNA-induced silencing complex (RISC) resulting in the specific cleavage of viral mRNA, thereby inhibiting lethal, acute infections and permitting virus persistence in the insect vector [34,35]. The siRNA defense system may also suppress the replication of mammalian arboviruses in their insect vectors to levels that favour the establishment of persistence and the survival of the vector [36]. However, it is not known whether other RNA viruses, such as certain paramyxoviruses, that can establish persistent infections have also evolved specific mechanisms to down regulate virus transcription and replication under certain conditions. Nevertheless, it is interesting to speculate that the reason some viruses can establish persistence in their natural hosts but cause serious disease in other species (*e.g.* hantaviruses [37,38], or possibly even Ebola virus), is because the mechanisms they have evolved to dampen replication in their natural hosts do not function in other species.

It is unlikely that highly cytotoxic viruses will be able to establish persistent infections unless either some infected cells are restrictive or semi-permissive for virus replication, or virus variants, including temperature sensitive mutants [39], with reduced cytopathogenicity are selected during the establishment of persistent infections. Such an outcome is much more likely for RNA viruses than DNA viruses given the high mutation rate of

RNA viruses and their quasispecies nature [40–43]. Similarly, the presence and amplification of defective interfering particles may also dampen virus replication and thus influence the establishment of persistence [44–46]. A rare way to establish RNA virus persistence, that is unlikely to be of evolutionary benefit to the virus, is the production of a cDNA copy of the viral RNA by endogenous reverse transcriptase, as has occurred for BDV and been proposed for measles virus and LCMV [47–50].

One general way for viruses to restrict replication is through limited activation of the interferon (IFN) response. IFNs are cellular factors produced by infected cells that interact with receptors on infected and uninfected cells to induce expression of antiviral proteins that restrict virus replication. This is an extremely powerful antiviral response that has co-evolved between virus and host for millions of years and is a programmed response that plays an essential role in controlling many virus infections. Regulation of this innate response plays a role in persistent hepatitis A infection of hepatocytes [51] and arbovirus infection of mature neurons [52]. There is also evidence that, for some viruses, variants that induce IFN, or are relatively sensitive to IFN, may be better able than the wild type viruses to establish persistent infections [53,54].

Interaction with the immune system

A major host factor that profoundly influences the establishment of persistent infections is the competence of the immune response and patients with immunodeficiencies in innate, adaptive, or combined immune responses are susceptible to development of persistent and progressive infections with attenuated as well as wild type RNA viruses. As examples, in addition to progressive disease in severely immunodeficient children [55], children with defects in the IFN response cannot rapidly clear the attenuated viruses included in the measles-mumps-rubella (MMR) vaccine, despite having an apparently normal adaptive immune response [56,57]. Polymorphisms in the IFN- λ response have been reported to influence the outcome of HCV and its ability to establish persistent infections [58]. Individuals with an inability to make immunoglobulins (agammaglobulinemia) can become persistently infected with a variety of RNA viruses, including echoviruses, enteroviruses, rhinoviruses and parainfluenza viruses [11,59–61]. Furthermore, immunodeficient individuals persistently infected with poliovirus are a challenge for the WHO's vaccination campaign to eradicate poliomyelitis [62]. Indeed, given our advances in how to treat autoimmune disease with immunosuppressive drugs and the survival of patients with immunodeficient disorders, such individuals, if they become persistently infected, may become significant reservoirs for some infectious diseases and nosocomial infections.

To establish persistent infections in immunocompetent individuals, viruses must avoid elimination by a fully functional immune system, including innate and adaptive responses. With regards innate responses, these include avoidance of elimination by apoptosis [63] and the IFN response. Indeed, to survive in nature all viruses must at least partially circumvent the IFN response and to do so they often hide, or modify their genomes, such that they do not activate the IFN system, and/or produce proteins that act as IFN antagonists [64]. The mechanisms by which these IFN antagonists work potentially have a strong influence on the ability of viruses to establish persistent infections. Some lytic viruses (*e.g.* alpha-viruses in vertebrate hosts) block the IFN response by inhibiting cellular transcription or protein synthesis, which inevitably leads to cell death. Therefore, for reasons discussed above, it is likely that non-cytolytic variants will evolve during the establishment of persistent infections. Other RNA viruses by nature are less lytic, and produce IFN antagonists that allow cell survival and promote the establishment of virus persistence. Indeed, the mechanisms of action of such viral IFN antagonists may have specifically evolved to facilitate persistence.

To establish persistence viruses must also avoid elimination by antibody and T-cell responses, and this may necessitate the down-regulation of virus protein synthesis and replication (see above). Viruses may also establish persistent infection in immunologically privileged sites such as the brain or testis [5,65,66] and, although the brain is likely to be a dead-end organ for the most viruses, testicular infections can facilitate transmission [66–69]. The likelihood of persistence can also be influenced by the promptness of the virus-specific antibody response and relative timing of the appearance of populations of effector and regulatory T cells. Viruses may be more likely to persist if the effector response is suppressed prior to clearance or if infection occurs at a very young age and immunologic dysfunction/tolerance is established (*e.g.* bovine viral diarrhoea virus infection in cattle, congenital rubella in humans, LCMV in mice) [70–72]. Viruses can also suppress development of the adaptive antiviral immune response and promote persistence by replicating in cells and tissues of the immune system [73,74]. For instance, bluetongue virus, an arthropod-borne reovirus, infects and destroys follicular dendritic cells in germinal centres of lymph nodes and prevents prompt development of antibody capable of clearing infection [75]. Conversely it has also been suggested that an inappropriate antibody response may facilitate persistence, as has been suggested for MeV and Junin virus infections [76,77]. How this may work is an open question, but antibody-induced antigenic modulation can reduce the level of measles viral glycoproteins on the surface of persistently infected cells below that required for lysis by antibody-dependent cellular cytotoxicity (ADCC), a

mechanism by which specific immune cells, including natural killer cells and macrophages, target virus-infected cells [78]. Furthermore, antibodies against the measles virus haemagglutinin can also reduce the expression of viral proteins within cells, thereby potentially preventing cell death and reducing the likelihood of the cells being killed by the immune response [79,80].

To conclude, despite the fact that persistent RNA virus infections can have important consequences both for the virus and host, much has still to be learned about ‘Within host persistent RNA virus’ infections. With the advent of new technologies, such as next generation sequencing, the tools are now available to better study persistent infections both *in vivo* and *in vitro*. Such studies may be used to investigate possible associations of persistent virus infections with chronic human diseases. A better understanding of the incidence and nature of persistent RNA virus infections may also promote development of better methods for surveillance and control; for example, it may be possible to design improved vaccines using viruses that establish persistent infections as vectors to induce long lasting immunity.

Acknowledgments

RER is funded by the Wellcome Trust, UK (Grant 101788/Z/13/Z) and DEG by US National Institutes of Health (R01 NS038932). The University of St Andrews is a charity registered in Scotland (SC013532).

References

- Anderson RM, May RM: **Immunisation and herd immunity.** *Lancet* 1990, **335**:641-645.
- Nathanson N, Moss WJ: **Epidemiology.** In *Fields Virology*. Edited by Knipe DM, Howley PM. Lippincott, Williams & Wilkins; 2013: 483-507.
- Moonen P, Schrijver R: **Carriers of foot-and-mouth disease virus: a review.** *Vet Q* 2000, **22**:193-197.
- Salt JS: **The carrier state in foot and mouth disease—an immunological review.** *Br Vet J* 1993, **149**:207-223.
- Kristensson K, Norrby E: **Persistence of RNA viruses in the central nervous system.** *Annu Rev Microbiol* 1986, **40**:159-184.
- Garg RK: **Subacute sclerosing panencephalitis.** *Postgrad Med J* 2002, **78**:63-70.
- Griffin DE, Lin WH, Pan CH: **Measles virus, immune control, and persistence.** *FEMS Microbiol Rev* 2012, **36**:649-662.
- Lin F, Mackay DK, Knowles NJ: **The persistence of swine vesicular disease virus infection in pigs.** *Epidemiol Infect* 1998, **121**:459-472.
- Zlateva KT, de Vries JJ, Coenjaerts FE, van Loon AM, Verheij T, Little P, Butler CC, Goossens H, Ieven M, Claas EC *et al.*: **Prolonged shedding of rhinovirus and re-infection in adults with respiratory tract illness.** *Eur Respir J* 2014, **44**:169-177.
- Loeffelholz MJ, Trujillo R, Pyles RB, Miller AL, Alvarez-Fernandez P, Pong DL, Chonmaitree T: **Duration of rhinovirus shedding in the upper respiratory tract in the first year of life.** *Pediatrics* 2014, **134**:1144-1150.
- Randall RE, Russell WC: **Paramyxovirus persistence. Consequences for host and virus.** In *The Paramyxoviruses*. Edited by Kingsbury DW. Plenum Press; 1991:299-321.

12. Nuttall PA, Jones LD, Labuda M, Kaufman WR: **Adaptations of arboviruses to ticks.** *J Med Entomol* 1994, **31**:1-9.
13. Kuno G: **Persistence of arboviruses and antiviral antibodies in vertebrate hosts: its occurrence and impacts.** *Rev Med Virol* 2001, **11**:165-190.
14. Heeney JL: **Ebola hidden reservoirs.** *Nature* 2015, **527**:453-455.
15. Harrower J, Kiedrzyński T, Baker S, Upton A, Rahnama F, Sherwood J, Huang QS, Todd A, Pulford D: **Sexual transmission of Zika virus and persistence in Semen, New Zealand, 2016.** *Emerg Infect Dis* 2016, **22**:1855-1857.
16. Lin WH, Kouyos RD, Adams RJ, Grenfell BT, Griffin DE: **Prolonged persistence of measles virus RNA is characteristic of primary infection dynamics.** *Proc Natl Acad Sci U S A* 2012, **109**:14989-14994.
17. Panum PL: **lagttagaiser anstillede under maeslingeapidemieu paa faeroernei aaret 1846.** *Bibliothek Laeger* 1847, **1**:270-344.
18. McGivern DR, Lemon SM: **Virus-specific mechanisms of carcinogenesis in hepatitis C virus associated liver cancer.** *Oncogene* 2011, **30**:1969-1983.
19. Miller KD, Schnell MJ, Rall GF: **Keeping it in check: chronic viral infection and antiviral immunity in the brain.** *Nat Rev Neurosci* 2016, **17**:766-776.
20. Jacobs M, Rodger A, Bell DJ, Bhagani S, Cropley I, Filipe A, Gifford RJ, Hopkins S, Hughes J, Jabeen F *et al.*: **Late Ebola virus relapse causing meningoencephalitis: a case report.** *Lancet* 2016, **388**:498-503.
21. MacLachlan NJ, Nunamaker RA, Katz JB, Sawyer MM, Akita GY, Osburn BI, Tabachnick WJ: **Detection of bluetongue virus in the blood of inoculated calves: comparison of virus isolation, PCR assay, and in vitro feeding of *Culicoides variipennis*.** *Arch Virol* 1994, **136**:1-8.
22. Schwartz-Cornil I, Mertens PP, Contreras V, Hemati B, Pascale F, Breard E, Mellor PS, MacLachlan NJ, Zientara S: **Bluetongue virus: virology, pathogenesis and immunity.** *Vet Res* 2008, **39**:46.
23. Whetter LE, MacLachlan NJ, Gebhard DH, Heidner HW, Moore PF: **Bluetongue virus infection of bovine monocytes.** *J Gen Virol* 1989, **70**(Pt. 7):1663-1676.
24. Li Y, Masaki T, Yamane D, McGivern DR, Lemon SM: **Competing and noncompeting activities of miR-122 and the 5' exonuclease Xrn1 in regulation of hepatitis C virus replication.** *Proc Natl Acad Sci U S A* 2013, **110**:1881-1886.
25. Sarnow P, Sagan SM: **Unraveling the mysterious interactions between hepatitis C virus RNA and liver-specific microRNA-122.** *Annu Rev Virol* 2016, **3**:309-332.
26. Tomonaga K, Kobayashi T, Ikuta K: **Molecular and cellular biology of Borna disease virus infection.** *Microbes Infect* 2002, **4**:491-500.
27. Matsumoto Y, Hayashi Y, Omori H, Honda T, Daito T, Horie M, Ikuta K, Fujino K, Nakamura S, Schneider U *et al.*: **Bornavirus closely associates and segregates with host chromosomes to ensure persistent intranuclear infection.** *Cell Host Microbe* 2012, **11**:492-503.
28. Poenisch M, Burger N, Staeheli P, Bauer G, Schneider U: **Protein X of Borna disease virus inhibits apoptosis and promotes viral persistence in the central nervous systems of newborn-infected rats.** *J Virol* 2009, **83**:4297-4307.
29. Schneider U: **Novel insights into the regulation of the viral polymerase complex of neurotropic Borna disease virus.** *Virus Res* 2005, **111**:148-160.
30. Schneider U, Martin A, Schwemmler M, Staeheli P: **Genome trimming by Borna disease viruses: viral replication control or escape from cellular surveillance.** *Cell Mol Life Sci* 2007, **64**:1038-1042.
31. Kim KS, Tracy S, Tappich W, Bailey J, Lee CK, Kim K, Barry WH, Chapman NM: **5'-terminal deletions occur in coxsackievirus B3 during replication in murine hearts and cardiac myocyte cultures and correlate with encapsidation of negative-strand viral RNA.** *J Virol* 2005, **79**:7024-7041.
32. Meyer BJ, Southern PJ: **A novel type of defective viral genome suggests a unique strategy to establish and maintain persistent lymphocytic choriomeningitis virus infections.** *J Virol* 1997, **71**:6757-6764.
33. Meyer BJ, Schmaljohn CS: **Persistent hantavirus infections: characteristics and mechanisms.** *Trends Microbiol* 2000, **8**:61-67.
34. Lan H, Wang H, Chen Q, Chen H, Jia D, Mao Q, Wei T: **Small interfering RNA pathway modulates persistent infection of a plant virus in its insect vector.** *Sci Rep* 2016, **6**:20699.
35. Goic B, Vodovar N, Mondotte JA, Monot C, Frangeul L, Blanc H, Gausson V, Vera-Otarola J, Cristofari G, Saleh MC: **RNA-mediated interference and reverse transcription control the persistence of RNA viruses in the insect model *Drosophila*.** *Nat Immunol* 2013, **14**:396-403.
36. Myles KM, Wiley MR, Morazzani EM, Adelman ZN: **Alphavirus-derived small RNAs modulate pathogenesis in disease vector mosquitoes.** *Proc Natl Acad Sci U S A* 2008, **105**:19938-19943.
37. Klein SL, Calisher CH: **Emergence and persistence of hantaviruses.** *Curr Top Microbiol Immunol* 2007, **315**:217-252.
38. Ermonval M, Baychelier F, Tordo N: **What do we know about how Hantaviruses interact with their different hosts?** *Viruses* 2016, **8**.
39. Younger JS: **Temperature-sensitive viruses: possible role in chronic and inapparent infections.** In *Slow Virus Infections of the Central Nervous Systems*. Edited by ter Meulen V, Katz M. Springer-Verlag; 1977:222-227.
40. Vignuzzi M, Stone JK, Arnold JJ, Cameron CE, Andino R: **Quasispecies diversity determines pathogenesis through cooperative interactions in a viral population.** *Nature* 2006, **439**:344-348.
41. Sanz-Ramos M, Diaz-San Segundo F, Escarmis C, Domingo E, Sevilla N: **Hidden virulence determinants in a viral quasispecies in vivo.** *J Virol* 2008, **82**:10465-10476.
42. Domingo E, Baranowski E, Ruiz-Jarabo CM, Martin-Hernandez AM, Saiz JC, Escarmis C: **Quasispecies structure and persistence of RNA viruses.** *Emerg Infect Dis* 1998, **4**:521-527.
43. Domingo E, Sheldon J, Perales C: **Viral quasispecies evolution.** *Microbiol Mol Biol Rev* 2012, **76**:159-216.
44. Holland JJ, Kennedy IT, Semler BL, Jones CL, Roux L, Grabau EA: **Defective interfering RNA viruses and the host-cell response.** In *Comprehensive Virology*, vol. 16. Edited by Fraenkel-Conrat H, Wagner RR. Plenum Press; 1980:137-192.
45. Roux L, Simon AE, Holland JJ: **Effects of defective interfering viruses on virus replication and pathogenesis in vitro and in vivo.** *Adv Virus Res* 1991, **40**:181-211.
46. Sidhu MS, Crowley J, Lowenthal A, Karcher D, Menonna J, Cook S, Udem S, Dowling P: **Defective measles virus in human subacute sclerosing panencephalitis brain.** *Virology* 1994, **202**:631-641.
47. Zhdanov VM: **Integration of viral genomes.** *Nature* 1975, **256**:471-473.
48. Geuking MB, Weber J, Dewannieux M, Gorelik E, Heidmann T, Hengartner H, Zinkernagel RM, Hangartner L: **Recombination of retrotransposon and exogenous RNA virus results in nonretroviral cDNA integration.** *Science* 2009, **323**:393-396.
49. Klenerman P, Hengartner H, Zinkernagel RM: **A non-retroviral RNA virus persists in DNA form.** *Nature* 1997, **390**:298-301.
50. Horie M, Honda T, Suzuki Y, Kobayashi Y, Daito T, Oshida T, Ikuta K, Jern P, Gojobori T, Coffin JM *et al.*: **Endogenous non-retroviral RNA virus elements in mammalian genomes.** *Nature* 2010, **463**:84-87.
51. Lanford RE, Feng Z, Chavez D, Guerra B, Brasky KM, Zhou Y, Yamane D, Perelson AS, Walker CM, Lemon SM: **Acute hepatitis**

- A virus infection is associated with a limited type I interferon response and persistence of intrahepatic viral RNA.** *Proc Natl Acad Sci U S A* 2011, **108**:11223-11228.
52. Schultz KL, Vernon PS, Griffin DE: **Differentiation of neurons restricts Arbovirus replication and increases expression of the alpha isoform of IRF-7.** *J Virol* 2015, **89**:48-60.
 53. Chatziandreu N, Young D, Andrejeva J, Goodbourn S, Randall RE: **Differences in interferon sensitivity and biological properties of two related isolates of simian virus 5: a model for virus persistence.** *Virology* 2002, **293**:234-242.
 54. Sekellick MJ, Marcus PI: **Persistent infection. II. Interferon-inducing temperature-sensitive mutants as mediators of cell sparing: possible role in persistent infection by vesicular stomatitis virus.** *Virology* 1979, **95**:36-47.
 55. Bitnun A, Shannon P, Durward A, Rota PA, Bellini WJ, Graham C, Wang E, Ford-Jones EL, Cox P, Becker L *et al.*: **Measles inclusion-body encephalitis caused by the vaccine strain of measles virus.** *Clin Infect Dis* 1999, **29**:855-861.
 56. Hambleton S, Goodbourn S, Young DF, Dickinson P, Mohamad SM, Valappil M, McGovern N, Cant AJ, Hackett SJ, Ghazal P *et al.*: **STAT2 deficiency and susceptibility to viral illness in humans.** *Proc Natl Acad Sci U S A* 2013, **110**:3053-3058.
 57. Duncan CJA, Mohamad SMB, Young DA, Skelton AJ, Leahy TR, Munday DC, Butler KM, Morfopoulou S, Brown JR, Hubank M, Connell J, Gavin PJ, McMahon C, Dempsey E, Lynch NE, Jacques TS, Valappil M, Cant AJ, Engelhardt K, Breuer J, Randall RE, Hambleton S: **Human IFNAR2 deficiency: lessons for antiviral immunity.** *Sci Transl Med* 2015, **7**:307ra154.
 58. Laidlaw SM, Dustin LB: **Interferon lambda: opportunities, risks, and uncertainties in the fight against HCV.** *Front Immunol* 2014, **5**:545.
 59. Kainulainen L, Vuorinen T, Rantakokko-Jalava K, Osterback R, Ruuskanen O: **Recurrent and persistent respiratory tract viral infections in patients with primary hypogammaglobulinemia.** *J Allergy Clin Immunol* 2010, **126**:120-126.
 60. Peltola V, Waris M, Kainulainen L, Kero J, Ruuskanen O: **Virus shedding after human rhinovirus infection in children, adults and patients with hypogammaglobulinaemia.** *Clin Microbiol Infect* 2013, **19**:E322-327.
 61. Wilfert CM, Buckley RH, Mohanakumar T, Griffith JF, Katz SL, Whisnant JK, Eggleston PA, Moore M, Treadwell E, Oxman MN *et al.*: **Persistent and fatal central-nervous-system ECHOvirus infections in patients with agammaglobulinemia.** *N Engl J Med* 1977, **296**:1485-1489.
 62. Dunn G, Klapsa D, Wilton T, Stone L, Minor PD, Martin J: **Twenty-eight years of poliovirus replication in an immunodeficient individual: impact on the global polio eradication initiative.** *PLoS Pathog* 2015, **11**:e1005114.
 63. Roulston A, Marcellus RC, Branton PE: **Viruses and apoptosis.** *Annu Rev Microbiol* 1999, **53**:577-628.
 64. Randall RE, Goodbourn S: **Interferons and viruses: an interplay between induction, signalling, antiviral responses and virus countermeasures.** *J Gen Virol* 2008, **89**:1-47.
 65. Griffin DE: **Immune responses to RNA-virus infections of the CNS.** *Nat Rev Immunol* 2003, **3**:493-502.
 66. Dejuco N, Jegou B: **Viruses in the mammalian male genital tract and their effects on the reproductive system.** *Microbiol Mol Biol Rev* 2001, **65**:208-231.
 67. Petersen LR, Jamieson DJ, Powers AM, Honein MA: **Zika virus.** *N Engl J Med* 2016, **374**:1552-1563.
 68. Crozier I: **Ebola virus RNA in the semen of male survivors of Ebola virus disease: the uncertain gravitas of a privileged persistence.** *J Infect Dis* 2016, **214**:1467-1469.
 69. Deen GF, Knust B, Broutet N, Sesay FR, Formenty P, Ross C, Thorson AE, Massaquoi TA, Marrinan JE, Ervin E *et al.*: **Ebola RNA persistence in semen of ebola virus disease survivors—preliminary report.** *N Engl J Med* 2015:1-7.
 70. Peterhans E, Schweizer M: **Pestiviruses: how to outmaneuver your hosts.** *Vet Microbiol* 2010, **142**:18-25.
 71. Oldstone MB: **Anatomy of viral persistence.** *PLoS Pathog* 2009, **5**:e1000523.
 72. Fuccillo DA, Steele RW, Hensen SA, Vincent MM, Hardy JB, Bellanti JA: **Impaired cellular immunity to rubella virus in congenital rubella.** *Infect Immun* 1974, **9**:81-84.
 73. Oldstone MB: **Viral persistence.** *Cell* 1989, **56**:517-520.
 74. Borrow P: **Mechanisms of viral clearance and persistence.** *J Viral Hepat* 1997, **4**(Suppl. 2):16-24.
 75. Melzi E, Caporale M, Rocchi M, Martin V, Gamino V, di Provvido A, Marruchella G, Entrican G, Sevilla N, Palmarini M: **Follicular dendritic cell disruption as a novel mechanism of virus-induced immunosuppression.** *Proc Natl Acad Sci U S A* 2016, **113**:E6238-E6247.
 76. Rammohan KW, McFarland HF, McFarlin DE: **Subacute sclerosing panencephalitis after passive immunization and natural measles infection: role of antibody in persistence of measles virus.** *Neurology* 1982, **32**:390-394.
 77. Maiztegui JI, Fernandez NJ, de Damilano AJ: **Efficacy of immune plasma in treatment of Argentine haemorrhagic fever and association between treatment and a late neurological syndrome.** *Lancet* 1979, **2**:1216-1217.
 78. Oldstone MBA, Fujinami RS: **Virus persistence and avoidance of immune surveillance: how measles can be induced to persist in cells, escape immune assault and injure tissues.** In *Virus Persistence*. Edited by Mahy BW, Minson AC, Darby GK. Cambridge University Press; 1982:185-202.
 79. Fujinami RS, Oldstone MB: **Antigenic modulation: a mechanism of viral persistence.** *Prog Brain Res* 1983, **59**:105-111.
 80. Fujinami RS, Oldstone MB: **Antiviral antibody reacting on the plasma membrane alters measles virus expression inside the cell.** *Nature* 1979, **279**:529-530.
 81. Buchmeier MJ, de la Torre J, Peters CJ: **Arenaviridae: the viruses and their replication.** In *Fields Virology*. Edited by Knipe DM, Howley DM. Lippincott Williams & Wilkins; 2007:1791-1828.
 82. Snijder EJ, Kikkert M, Fang Y: **Arterivirus molecular biology and pathogenesis.** *J Gen Virol* 2013, **94**:2141-2163.
 83. de la Torre JC: **Molecular biology of Borna disease virus and persistence.** *Front Biosci* 2002, **7**:d569-d579.
 84. Abbott KD, Ksiazek TG, Mills JN: **Long-term hantavirus persistence in rodent populations in central Arizona.** *Emerg Infect Dis* 1999, **5**:102-112.
 85. Sukhrie FH, Siebenga JJ, Beersma MF, Koopmans M: **Chronic shedders as reservoir for nosocomial transmission of norovirus.** *J Clin Microbiol* 2010, **48**:4303-4305.
 86. Bergmann CC, Lane TE, Stohman SA: **Coronavirus infection of the central nervous system: host-virus stand-off.** *Nat Rev Microbiol* 2006, **4**:121-132.
 87. Roossinck MJ: **Persistent plant viruses: molecular hitchhikers or epigenetic elements?** In *Viruses: Essential Agents of Life*. Edited by Witzany G. Springer; 2012:177-186.
 88. Gear JS, Cassel GA, Gear AJ, Trappler B, Clausen L, Meyers AM, Kew MC, Bothwell TH, Sher R, Miller GB *et al.*: **Outbreak of Marburg virus disease in Johannesburg.** *Br Med J* 1975, **4**:489-493.
 89. Memon MI, Memon MA: **Hepatitis C: an epidemiological review.** *J Viral Hepat* 2002, **9**:84-100.
 90. Stapleton JT, Fong S, Muerhoff AS, Bukh J, Simmonds P: **The GB viruses: a review and proposed classification of GBV-A, GBV-C (HGV), and GBV-D in genus Pegivirus within the family Flaviviridae.** *J Gen Virol* 2011, **92**:233-246.
 91. Komar N, Langevin S, Hinten S, Nemeth N, Edwards E, Hettler D, Davis B, Bowen R, Bunning M: **Experimental infection of North American birds with the New York 1999 strain of West Nile virus.** *Emerg Infect Dis* 2003, **9**:311-322.

92. Ricklin ME, Garcia-Nicolas O, Brechbuhl D, Python S, Zumkehr B, Nougaiere A, Charrel RN, Posthaus H, Oevermann A, Summerfield A: **Vector-free transmission and persistence of Japanese encephalitis virus in pigs.** *Nat Commun* 2016, **7**:10832.
93. Gritsun TS, Lashkevich VA, Gould EA: **Tick-borne encephalitis.** *Antiviral Res* 2003, **57**:129-146.
94. Peterhans E, Jungi TW, Schweizer M: **BVDV and innate immunity.** *Biologicals* 2003, **31**:107-112.
95. Nettleton PF, Entrican G: **Ruminant pestiviruses.** *Br Vet J* 1995, **151**:615-642.
96. Dasgupta R, Free HM, Zietlow SL, Paskewitz SM, Aksoy S, Shi L, Fuchs J, Hu C, Christensen BM: **Replication of flock house virus in three genera of medically important insects.** *J Med Entomol* 2007, **44**:102-110.
97. Pinsky BA, Mix S, Rowe J, Ikemoto S, Baron EJ: **Long-term shedding of influenza A virus in stool of immunocompromised child.** *Emerg Infect Dis* 2010, **16**:1165-1167.
98. Olsen B, Munster VJ, Wallensten A, Waldenstrom J, Osterhaus AD, Fouchier RA: **Global patterns of influenza A virus in wild birds.** *Science* 2006, **312**:384-388.
99. Rima BK, Duprex WP: **Molecular mechanisms of measles virus persistence.** *Virus Res* 2005, **111**:132-147.
100. Axthelm MK, Krakowka S: **Experimental old dog encephalitis (ODE) in a gnotobiotic dog.** *Vet Pathol* 1998, **35**:527-534.
101. Sharp CR, Nambulli S, Acciardo AS, Rennick LJ, Drexler JF, Rima BK, Williams T, Duprex WP: **Chronic infection of domestic cats with feline morbillivirus, United States.** *Emerg Infect Dis* 2016, **22**:760-762.
102. Vandeveld M, Zurbriggen A: **Demyelination in canine distemper virus infection: a review.** *Acta Neuropathol* 2005, **109**:56-68.
103. Muchmore HG, Parkinson AJ, Humphries JE, Scott EN, McIntosh DA, Scott LV, Cooney MK, Miles JA: **Persistent parainfluenza virus shedding during isolation at the South Pole.** *Nature* 1981, **289**:187-189.
104. Atoyntan T, Hsiung GD: **Epidemiologic studies of latent virus infections in captive monkeys and baboons. II. Serologic evidence of myxovirus infections with special reference to SV5.** *Am J Epidemiol* 1969, **89**:472-479.
105. Cuevas-Romero S, Hernandez-Baumgarten E, Kennedy S, Hernandez-Jauregui P, Berg M, Moreno-Lopez J: **Long-term RNA persistence of porcine rubulavirus (PorPV-LPMV) after an outbreak of a natural infection: the detection of viral mRNA in sentinel pigs suggests viral transmission.** *Virus Res* 2014, **188**:155-161.
106. Brahic M: **Theiler's virus infection of the mouse, or: of the importance of studying animal models.** *Virology* 2002, **301**:1-5.
107. Schwarze J, O'Donnell DR, Rohwedder A, Openshaw PJ: **Latency and persistence of respiratory syncytial virus despite T cell immunity.** *Am J Respir Crit Care Med* 2004, **169**:801-805.
108. Sikkil MB, Quint JK, Mallia P, Wedzicha JA, Johnston SL: **Respiratory syncytial virus persistence in chronic obstructive pulmonary disease.** *Pediatr Infect Dis J* 2008, **27**:S63-S70.
109. Gomez B: **Respiratory syncytial virus persistence.** *Virology* 2012, **1**:1-4.
110. Bingham J: **Canine rabies ecology in southern Africa.** *Emerg Infect Dis* 2005, **11**:1337-1342.
111. Banyard AC, Hayman D, Johnson N, McElhinney L, Fooks AR: **Bats and lyssaviruses.** *Adv Virus Res* 2011, **79**:239-289.
112. Labadie K, Larcher T, Joubert C, Mannioui A, Delache B, Brochard P, Guigand L, Dubreil L, Lebon P, Verrier B et al.: **Chikungunya disease in nonhuman primates involves long-term viral persistence in macrophages.** *J Clin Invest* 2010, **120**:894-906.
113. Weil ML, Itabashi H, Cremer NE, Oshiro L, Lennette EH, Carnay L: **Chronic progressive panencephalitis due to rubella virus simulating subacute sclerosing panencephalitis.** *N Engl J Med* 1975, **292**:994-998.
114. Fujinami RS, von Herrath MG, Christen U, Whitton JL: **Molecular mimicry, bystander activation, or viral persistence: infections and autoimmune disease.** *Clin Microbiol Rev* 2006, **19**:80-94.
115. Oldstone MB: **Molecular mimicry and immune-mediated diseases.** *FASEB J* 1998, **12**:1255-1265.
116. Chia JK: **The role of enterovirus in chronic fatigue syndrome.** *J Clin Pathol* 2005, **58**:1126-1132.
117. Di Bisceglie AM, Goodman ZD, Ishak KG, Hoofnagle JH, Melpolder JJ, Alter HJ: **Long-term clinical and histopathological follow-up of chronic posttransfusion hepatitis.** *Hepatology* 1991, **14**:969-974.
118. Lipton HL, Liang Z, Hertzler S, Son KN: **A specific viral cause of multiple sclerosis: one virus, one disease.** *Ann Neurol* 2007, **61**:514-523.
119. Virtanen JO, Jacobson S: **Viruses and multiple sclerosis.** *CNS Neurol Disord Drug Targets* 2012, **11**:528-544.
120. Roodman GD, Windle JJ: **Paget disease of bone.** *J Clin Invest* 2005, **115**:200-208.
121. Burt FJ, Rolph MS, Rulli NE, Mahalingam S, Heise MT: **Chikungunya: a re-emerging virus.** *Lancet* 2012, **379**:662-671.
122. Julien J, Leparac-Goffart I, Lina B, Fuchs F, Foray S, Janatova I, Aymard M, Kopecka H: **Postpolio syndrome: poliovirus persistence is involved in the pathogenesis.** *J Neurol* 1999, **246**:472-476.
123. Buchanan R, Bonthius DJ: **Measles virus and associated central nervous system sequelae.** *Semin Pediatr Neurol* 2012, **19**:107-114.
124. Wang JH, Kwon HJ, Jang YJ: **Detection of parainfluenza virus 3 in turbinate epithelial cells of postviral olfactory dysfunction patients.** *Laryngoscope* 2007, **117**:1445-1449.
125. Niedermeyer HP, Arnold W, Neubert WJ, Sedlmeier R: **Persistent measles virus infection as a possible cause of otosclerosis: state of the art.** *Ear Nose Throat J* 2000, **79**:552-554 556, 558 passim.
126. Komune N, Ohashi M, Matsumoto N, Kimitsuki T, Komune S, Yanagi Y: **No evidence for an association between persistent measles virus infection and otosclerosis among patients with otosclerosis in Japan.** *J Clin Microbiol* 2012, **50**:626-632.