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Persistent RNA virus infections: do PAMPS drive chronic disease?

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Abstract

Chronic disease associated with persistent RNA virus infections represents a key public health concern. While human immunodeficiency virus-1 and hepatitis C virus are perhaps the most well-known examples of persistent RNA viruses that cause chronic disease, evidence suggests that many other RNA viruses, including re-emerging viruses such as chikungunya virus, Ebola virus and Zika virus, establish persistent infections. The mechanisms by which RNA viruses drive chronic disease are poorly understood. Here, we discuss how the persistence of viral RNA may drive chronic disease manifestations via the activation of RNA sensing pathways.

Introduction

Human diseases associated with persistent RNA virus infections are a major public health burden. Millions of people are persistently infected with human immunodeficiency virus-1 (HIV-1) and hepatitis C virus (HCV), and these persistent infections lead to serious health complications including acquired immune deficiency syndrome (AIDS), or liver fibrosis and failure, and hepatocellular carcinoma (HCC), respectively. In addition to these prominent examples of persistent RNA virus infections, increasing evidence suggests the numerous other RNA viruses, including some unexpected new players such as Ebola virus (EBOV) and Zika virus (ZIKV), establish persistence. Increasing efforts are needed to understand the mechanisms by which RNA viruses persist in hosts and to unravel how these persistent infections, or the persistence of viral products, contribute to human disease.

RNA virus persistence and chronic human disease

Many factors contribute to RNA virus persistence—that is, the persistence of infectious virus or viral products after acute infection. These include high viral diversity generated by error prone RNA polymerases and specific immune evasion mechanisms. In addition, certain body sites (e.g., the reproductive tract, ocular tissue, and the central nervous system [CNS]) are considered to be immune privileged - that is, these tissues have specialized regulatory

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mechanisms that influence the activation and effector function of the immune response to foreign antigens at these sites [1,2]. Due to these properties, several RNA viruses have been shown to persist in these tissues. However, other body tissues such as the lungs, myocardium, and joints, which are not generally considered prototypical immune privileged sites, are also associated with persistence of various RNA viruses (Figure 1).

It is well-established that the RNA viruses HIV-1 and HCV successfully establish and maintain persistent infections, and that these chronic infections cause chronic human disease. The causality between persistent infection and chronic disease is supported by the fact that direct-acting antivirals are effective treatments for the chronic disease manifestations associated with chronic HIV-1 and HCV infection. But what is the evidence that other RNA viruses establish persistent infections and that viral persistence contributes to chronic human disease?

Several RNA viruses persist in immune privileged tissues, and persistence is often accompanied by signs of inflammation. The underlying mechanisms driving chronic inflammation—even in immune privileged sites—are poorly understood. Measles virus (MeV), a morbillivirus with a negative-sense RNA genome, is the causative agent of an acute disease characterized by fever, cough, conjunctivitis, and a maculopapular rash. Studies in children and nonhuman primates (NHPs) have shown that MeV RNA persists in multiple tissues, including the blood, respiratory tract, and CNS, after clearance of infectious virus [3,4]. Sub-acute sclerosing panencephalitis (SSPE) is a rare fatal condition associated with persistent MeV infection of the CNS that manifests years after acute illness. The pathogenesis of SSPE is poorly understood, however, MeV RNA has been detected in the brain of SSPE autopsy specimens and MeV strains isolated from SSPE cases have mutations that attenuate viral replication and facilitate immune evasion [5–7], likely contributing to the ability of MeV to persist in the CNS for years after acute infection.

The 2013–2016 outbreak of Ebola virus (EBOV), a filovirus with a negative-sense RNA genome, was unprecedented in magnitude with more than 28,000 suspected cases. The scale of this outbreak and the high number of survivors revealed many previously unknown aspects of EBOV infection. It is now understood that survivors of EBOV infection commonly experience serious long-term complications, including arthralgia, fatigue, hearing loss, neurological deficits, and uveitis [8–10]. In addition to these post-Ebola complications, accumulating evidence suggests that EBOV RNA persists for months after acute infection at multiple sites including semen, ocular fluid, and the cerebral spinal fluid (CSF) [11–14]. Although the full impact of EBOV persistence on post-Ebola complications remains to be elucidated, several case reports indicate that EBOV persistence can have devastating consequences. For example, a nurse working in Sierra Leone developed severe EBOV disease, and accordingly, received intensive treatment leading to clinical recovery and the absence of detectable EBOV RNA in plasma. Nine months later, this patient developed acute meningitis and had EBOV in the CSF which was nearly identical in sequence to the original infecting EBOV [15]. Uveitis appears to be another common sequela of EBOV infection, experienced by 18–34% of survivors [9,10]. A physician treated for severe EBOV disease in the United States developed unilateral uveitis nine weeks after the clearance of viremia, and infectious EBOV was isolated from the aqueous humor of the eye [13]. These examples

demonstrate that EBOV persistence at immune privileged sites can lead to severe relapses of disease months after acute infection.

West Nile virus (WNV), an arbovirus that is a member of the family *Flaviviridae*, causes fever (~20% of cases) and neuroinvasive/encephalitic disease (<1% of cases). More than half of WNV patients, even those who did not have severe acute illness, report experiencing long-term sequelae after infection, including weakness and cognitive deficits [16,17]. It is unclear if these long-term clinical sequelae are due to residual tissue damage that occurred during the acute illness, or whether WNV persistence in the CNS could be a contributing factor. WNV RNA and focal areas of inflammation persist in the CNS of some mice up to 4 months after acute infection [18]. In addition to persistence in an immune privileged site such as the CNS, in a hamster model of infection, persistent WNV infection was detected in the brain as well as the kidney for weeks, with persistent viruria [19,20]. Initial human studies suggested that previous WNV infection was independently associated with chronic kidney disease and may be shed in urine [21,22], however, follow-up studies failed to confirm these findings [23,24]; therefore, this aspect of WNV infection requires further study.

Zika virus (ZIKV) is an emerging flavivirus associated with Guillain-Barré syndrome in infected adults and severe congenital abnormalities in infants born to mothers infected during pregnancy [25]. Early evidence suggests that ZIKV can persist in immune privileged sites after acute infection. For example, ZIKV persists in the testis and epididymis of experimentally-infected male mice, causing extensive tissue damage that can affect fertility [26,27]. Although the extent to which this occurs in humans is currently unknown, hematospermia and prostatitis have been reported in ZIKV-infected men and long-term ZIKV persistence in the semen of infected men has been shown to contribute to viral transmission [28,29]. ZIKV RNA also has been detected in the aqueous humor of an adult patient with uveitis during the acute phase of illness [30], and conjunctivitis appears to be a common symptom of acute ZIKV infection [25]. The persistence of ZIKV RNA in eye tissue of immunodeficient adult mice suggests that the eye also has the potential to be a site of persistence in humans [31]. It is too early to tell whether ZIKV persistence is associated with chronic disease in humans, or whether persistence merely facilitates transmission. Nevertheless, the re-emergence of this arbovirus and its association with severe disease highlights the urgent need to understand the acute and potential chronic manifestations of virus-induced illness.

Some RNA viruses seem to persist in tissues that are not considered prototypical immune privileged sites (e.g., lungs, myocardium, and joints) and the mechanisms of persistence during these infections are less clear. The paramyxovirus respiratory syncytial virus (RSV) is a leading cause of bronchiolitis in infants and young children. RSV may also persist for months in lungs and airways following acute infection, and RSV infection is strongly associated with the development of long-term airway disease, such as asthma and chronic wheezing [32]. In experimental animal models, the persistence of RSV RNA is associated with chronic lung inflammation and RSV RNA copy numbers correlate with airway hyperreactivity [33,34]. Although RSV persistence in children has not been extensively

investigated, RSV RNA has been detected in ~25% of adult patients with stable COPD, and its presence is associated with higher levels of serum inflammatory markers [35].

Associations between coxsackievirus persistence in myocardium and dilated cardiomyopathy (DCM) and cardiac dysfunction are well established [36–38]. This does not require infectious virus production, as transfection of mutant coxsackievirus B3 (CVB3) cDNA that cannot form infectious progeny but can express viral genes induces cytopathic effects in myocytes [39]. Transgenic mice with cardiac-restricted expression of the same replication-defective CVB3 cDNA develop morphological changes in the heart that resemble DCM in humans [40]. The myocytopathic effects of this mutant CVB3 cDNA were likely due to expression of the CVB3 protease 2a, which directly cleaves the cytoskeletal protein dystrophin [41]. In support of this, cardiac-restricted expression of the CVB3 protease 2a alone is sufficient to cause DCM in mice [42]. It remains to be determined whether persistent coxsackievirus RNA (without expression of the protease) can also drive cardiac inflammation.

Increasing evidence indicates that several arboviruses persist in joint-associated tissue long after acute infection. Chikungunya virus (CHIKV) is a mosquito-transmitted alphavirus with a positive-sense RNA genome that causes epidemics of incapacitating musculoskeletal inflammatory disease in humans. Acute CHIKV infection is characterized by a high fever with severe pain, inflammation, and swelling in joints. Up to two-thirds of infected individuals with acute CHIKV disease experience an incapacitating arthralgia, which often includes signs of joint inflammation and tenosynovitis, that persists for months or years after the acute phase [43–46]. Infection by related alphaviruses such as Mayaro, o'nyong'nyong, Ross River, and Sindbis viruses can also lead to chronic musculoskeletal disease in humans [47–50]. The mechanisms by which these viral infections lead to chronic disease remain poorly understood. However, CHIKV antigen and RNA have been detected in synovial and muscle tissue biopsies collected from patients suffering from chronic disease [51,52]. In addition, Ross River virus RNA has been detected in knee biopsies collected five weeks after the onset of joint symptoms [53]. In support of these data, long term persistence of CHIKV infection and chronic joint disease occurs in experimentally infected NHPs and mice [54–59]. Rubella virus, a related virus in the *Togaviridae* family, is also associated with chronic arthralgia and there is some evidence that rubella virus can persist in joint tissue [60,61]. These data suggest that persistence of togaviruses promotes a chronic inflammatory musculoskeletal disease.

Viral PAMPs - does vRNA drive chronic disease?

In many cases, the mechanisms by which persistent RNA virus infections contribute to chronic disease are not well understood. The presence of active inflammation and viral RNA (vRNA) during chronic disease states raises the possibility that ongoing disease is due to a persistent response to the vRNA itself. In other words, prolonged sensing of vRNA may promote inflammation and disease in tissues.

It is well-established that aberrant activation of RNA sensing pathways promotes inflammatory diseases in humans. Genetic disorders with mutations in genes associated with

cytosolic nucleic acid sensing and IFN signaling lead to chronic type I IFN upregulation and inflammatory disease [62]. In addition, both microbial and endogenous sources of nucleic acid have long been suggested as inflammatory triggers for rheumatic diseases including rheumatoid arthritis (RA). Chronic activation of Toll-like receptor (TLR) signaling by nucleic acid or endogenous damage-associated molecular patterns (DAMPs) is thought to play a major role in the pathogenesis of many inflammatory diseases, including RA and systemic lupus erythematosus [63]. TLRs 2, 3, 4, and 7 are highly expressed in synovial tissue of RA patients compared with patients with osteoarthritis or healthy controls, suggesting that inflamed joints in RA are hypersensitive to endogenous or microbial nucleic acids [64–66]. Additionally, dsRNA has been detected in the synovial fluid of RA patients, with significantly higher levels in patients with erosive disease [67]. Thus, it is possible that an acute viral infection could trigger sustained upregulation of TLRs or cytosolic RNA sensors, creating a cellular environment hypersensitive to the continual presence of vRNA and other signals of cellular damage.

As discussed above, evidence from human studies and experimental animal models suggests that chronic musculoskeletal disease following infection with various togaviruses is associated with the persistence of vRNA in joints. Interestingly, the intra-articular administration of viral dsRNA or the dsRNA mimic poly(I:C) alone induces arthritis in mice through IL-1R signaling [68]. The arthritogenic effect of dsRNA depends on type I IFN signaling, as *Ifnar1*^{-/-} mice are protected from dsRNA-induced arthritis and intra-articular injection of IFN- α alone causes arthritis [69]. Thus, injection of a viral PAMP alone or the downstream signal (type I IFN) is sufficient to induce disease in the absence of a replicating virus.

The sufficiency of dsRNA to induce arthritis in mice suggests that suppressing immune responses could alleviate post-CHIKV chronic arthralgia and synovitis, which is associated with the presence of vRNA [51,52,54–56,58] and persistently elevated type I IFN production [51,55]. Immunosuppressive therapy using methotrexate, corticosteroids, or TNF blockers has shown some efficacy, although not universal, at improving symptoms and limiting progressive joint damage in patients with chronic CHIKV disease [70,71]. The beneficial effects suggest that, at least in a subset of patients, ongoing disease is due to sustained inflammation, perhaps in response to the persistence of vRNA. Interestingly, persistent arthralgia and myalgia are the most common symptoms reported in EBOV survivors, and symptoms can last over two years after acute disease [8–10,72]. These long-lasting symptoms may share common mechanisms with those observed after CHIKV infection.

The protozoan parasite *Leishmania* and the endosymbiont *Leishmania* RNA virus 1 (LRV1), a dsRNA virus in the *Totiviridae* family, provide an interesting example of aberrant nucleic acid sensing in the promotion of a chronic inflammatory disease. Some *Leishmania* species cause local, disfiguring skin lesions (cutaneous leishmaniasis), while others can disseminate to mucosal tissues to cause severe, life-threatening lesions (mucocutaneous leishmaniasis). The presence of LRV1 in human leishmaniasis is associated with treatment failure and more severe cutaneous disease manifestations [73–75]. In mouse models, infection with *L. guyanensis* parasites that have high levels of LRV1 results in increased footpad swelling and parasite burdens [76]. Since extracellular spread of LRV1 in *Leishmania* is unlikely [77], the

host immune system primarily interacts with LRV1 proteins and nucleic acid when they are released into the host cytoplasm from dead parasites. Indeed, it was shown that host TLR3-mediated recognition of LRV1 dsRNA is largely responsible for the induction of hyperinflammation that worsens mucocutaneous leishmaniasis and promotes parasite persistence in macrophages [76–79]. To date, LRV1 is the only known example of a viral endosymbiont acting as an immunogen in its pathogen's host. However, it illustrates the ability of vRNA itself (through detection by TLR3) to drive an aberrant inflammatory response and chronic disease.

The progression to viral persistence often involves the acquisition of mutations that attenuate replication or lead to the production of defective viral genomes (DVGs). DVGs generated at the peak of viral replication during acute respiratory infection with RSV and Sendai virus are potent inducers of type I IFN [80–82]. Although packaging of DVGs and spread to new cells requires co-infection of a cell with a fully intact viral genome, many types of DVGs are capable of autonomous RNA replication. Only a small number of cells harboring full-length viral genome would be required to maintain persistent infection and the transmission of DVGs to new cells. Due to their shorter length, DVGs may be more efficiently transcribed over the full-length genome, leading to an overabundance of DVGs relative to full-length genomes. In this case, it is not production of small amounts of infectious virus that promote inflammation, but the DVG itself.

During chronic HCV infection, there is both ongoing productive replication and abundant production of deletion mutants (i.e., DVGs). HCV DVGs have been reported in the serum and liver biopsies of chronically infected patients [83–86]. Many of these HCV DVGs retain the sequences necessary for RNA replication and can be packaged into viral particles when present in a cell co-infected with wild-type virus [86]. Although the continued propagation of the DVGs depends on replication of the full-length virus, these DVGs are likely potent inducers of type I IFN and inflammation during chronic HCV infection. A recent study demonstrated an association between the presence of HCV deletion mutants in the serum of chronically infected patients and increased hepatic inflammation [87]. This association was independent of viremia, supporting the idea that persistent vRNA itself promotes inflammation and disease during a chronic viral infection.

Concluding Remarks

The public health burden of chronic disease due to RNA virus infection is substantial. The mechanisms by which many RNA viruses drive chronic disease are poorly understood and likely involve a complex interplay of factors including host genetic susceptibility and environment. The ongoing inflammatory response to persistent viral RNA likely contributes to chronic disease manifestations in many cases. More research is needed to understand mechanisms of disease underlying persistent RNA virus infections.

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Highlights

- Many persistent RNA virus infections are associated with chronic disease
- Chronic disease mechanisms in these infections are poorly understood
- Persistent viral RNA may drive chronic inflammation and disease in many cases

Figure 1. Tissues associated with RNA virus persistence

Several RNA viruses from diverse virus families persist in immune privileged body sites and tissues (green/blue boxes). The male reproductive tract has been shown to harbor persistent ZIKV and EBOV, contributing to continued transmission, and (in animal models of ZIKV) damaging tissue and potentially affecting fertility. EBOV and ZIKV also persist in ocular tissue, the latter thus far demonstrated in animal models. The CNS [brain and CSF] is a site for persistent WNV (only demonstrated in animal models) as well as EBOV and MeV. Other body tissues which are not considered prototypical immune privileged sites (orange boxes) are associated with persistence of various RNA viruses. HCV persists in the liver. The myocardium is a well-established site of persistence for enteroviruses. Viral RNA and antigen from the togaviruses CHIKV and Ross River virus persist in joint-associated tissue, and in animal models CHIKV has been shown to persist in lymphoid tissue. The kidney has been identified as a potential site of WNV persistence in animal models, although this remains a speculative site of persistence in humans. RNA from the paramyxoviruses MeV and RSV persists in the respiratory tract.