Assessing the potential function of ADAR1 in virus-associated sepsis

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1. ABSTRACT

Sepsis syndrome is a common and frequently fatal clinical condition. It is defined by the presence of both infection and an uncontrolled systemic inflammatory response. It represents a major, although largely unappreciated, healthcare problem worldwide. It is especially problematic in infants and toddlers who show markedly increased susceptibility to severe infections caused by various pathogens, including viruses. Viruses are important causative agents of sepsis. Host adenosine deaminase acting on RNA 1 (ADAR1) catalyzes adenosine to inosine (A-to-I) editing of RNA transcripts, thus changing viral RNAs and exerting antiviral and proviral effects. In addition, ADAR1 promotes viral replication by directly interacting with protein kinase R and suppressing its kinase activity. We here discuss the function of ADAR1 and its regulatory role in viral infection. Further, we establish the relationship between ADAR1 and virus-associated sepsis, thus providing an important basis for the development of novel therapeutic targets for the treatment of virus-associated sepsis.

2. INTRODUCTION

Sepsis is a clinical syndrome that is defined by the presence of both, infection and an uncontrolled systemic inflammatory response. Dysfunction of the immune system is a crucial factor contributing to the outcome of sepsis (1, 2). Immune function defects culminate in a markedly increased susceptibility of infants and some toddlers to severe infections caused by various microorganisms, particularly viruses and encapsulated bacteria. Sepsis is the leading cause of death in infants and children worldwide (3–5).

Adenosine deaminases acting on RNA (ADARs) are enzymes involved in a type of RNA editing where adenosine residues are converted to inosine residues (A-to-I RNA editing) in double-stranded RNAs (dsRNAs). The translation machinery subsequently reads inosine as guanosine, leading to recoding of genes and diversification of their functions (6). Thus, A-to-I editing of both cellular and viral RNA substrates may potentially alter the coding capacity and structure of RNA, which may modulate RNA function (7). As the most important member of the ADAR family, ADAR1 has been shown to play important roles in embryonic erythropoiesis, viral immune response, and RNA interference (RNAi) (8). In this review, we discuss the characteristics of ADAR1, including its RNA-editing activity and its ability to regulate RNAi. We highlight recent research into how ADAR1 plays both antiviral and proviral roles during viral infections. Furthermore, we elaborate on how viruses cause sepsis. Thus, using viruses as a link between sepsis and ADAR1, we argue that ADAR1 may regulate viral infections, and that it might constitute a new target for antiviral agents.

3. ADAR1

3.1. Characteristics of ADAR1

Three ADARs exist in vertebrates (ADAR1–3) and all of them target dsRNAs (9–12). Members of the *ADAR* gene family share common structural features, such as multiple dsRNA binding domains (dsRBDs) and a separate deaminase domain. Three dsRBDs are present in ADAR1, but only two dsRBDs are found in ADAR2 and ADAR3 (13). Mammalian ADAR1 and ADAR2 are ubiquitously expressed in many tissues, while the expression of ADAR3 is limited to the nervous system (14, 15). Even though the carboxyl-terminal catalytic domains and dsRBDs of ADAR1–3 are conserved, only ADAR1 and ADAR2 possess a demonstrable enzymatic activity (14, 16, 17). These enzymes probably evolved from tRNA-processing adenosine deaminases after the split between the Protozoa and Metazoa (12).

There are two *ADAR1* transcripts, *ADAR1-L* and *ADAR1-S*, but only the former can be induced by interferon (IFN)-α/β and IFN-γ (18). The IFN-inducible form of ADAR1-L is a 150-kDa protein, referred to as p150, which localizes to both the cytoplasm and the nucleus (19); the smaller, constitutively expressed form of ADAR1-S, referred to as p110, localizes predominantly to the nucleus (19). Inactivation of *ADAR1* results in an embryonic lethal phenotype because of widespread apoptosis (20, 21), indicating that ADAR1 is essential for life. Several features of the p150 protein, including its presence in the cytoplasm [the site of negative-stranded RNA viruse–measles virus (MV) replication] and its induction by IFN, make it a likely candidate enzyme responsible for generating biased hypermutations in the M gene that are associated with subacute sclerosing panencephalitis. Furthermore, the induction of ADAR1 in the central nervous system as a result of viral infection likely contributes to neuronal cell dysfunction because ADAR1 is essential for glutamate and serotonin receptor editing (7, 22, 23).

3.2. RNAi modulation by ADAR1

A-to-I RNA editing occurs most often in non-coding regions that contain repetitive elements, such as *Alu*, and long interspersed nuclear elements (24). The levels of RNAi-dependent, endogenous short RNAs, which are derived from loci enriched in inverted repeats and transposons, are dramatically up-regulated in *ADAR null* mutant worms, indicating that A-to-I editing of dsRNA regions of transcripts derived from these loci inhibits their entry into the RNAi silencing pathway (25). The antagonistic relationship between A-to-I editing and RNAi, i.e., the competitive inhibition of the RNAi pathway by A-to-I editing of dsRNA substrates (25), results in structural alterations of these dsRNAs (specifically, a reduction

in their double-strandedness). Editing of precursor microRNAs (pri-miRNAs) can inhibit their processing (26, 27) and suppress RNA-induced silencing complex (RISC) loading (28), leading to silencing of a different set of target genes by native and edited miRNAs (29).

In addition to the aforementioned studies that indicate an antagonistic relationship between A-to-I RNA editing and RNAi pathways, interaction between ADAR1 and RISC component proteins has also been demonstrated (30). ADAR1 interacts directly with Dicer in an RNA binding-independent manner. This promotes the processing of small interfering RNAs and miRNAs, RISC loading of miRNAs, and, consequently, silencing of target RNAs, thereby revealing a stimulatory, rather than antagonistic, role of ADAR1 in RNAi (30). In addition to forming homodimers, which is required for the A-to-I RNA editing activities of ADAR1 (31), one monomer of ADAR1 can also bind one molecule of Dicer. Thus, ADAR1 acts as an RNA editing enzyme or as a modulator of the RNAi machinery by forming two different types of complexes, ADAR1 homodimers and Dicer/ADAR1 heterodimers, respectively.

Nevertheless, the exact mechanism that determines the balance between these two functions requires further elaboration. It has been previously reported that ~20% of pri-miRNAs in the human brain are edited, which often inhibits their processing to mature miRNAs (26). Despite a significant increase in *adar1* expression in E11–12 wild-type mouse embryos, A-to-I editing of pri-miRNAs is limited during that developmental stage (13, 32). Thus, ADAR1 function may be skewed toward that of an RNAi regulator, rather than an RNA editor, in developing embryos.

4. THE DUAL ROLES OF ADAR1 IN REGULATING VIRAL INFECTIONS

Considering that ADAR1 possesses these specialities elaborated above, it's significant to dig out its function in regulating viral infections. As shown in Figure 1, these functions will be exhibited in details below.

4.1. The RNA editing activity of ADAR1 modulates viral infections

Host cells restrict viral replication by a myriad of mechanisms, the best known of which are those that are induced by type I IFNs (21, 33). ADAR1 is proposed to play a role in host defense mechanisms because its expression induced by IFNs and viral infections (34, 35). ADAR1-edited MV genomes were first described in cases of subacute sclerosing panencephalitis , a rare, chronic degenerative disease that occurs several years after MV infection (36–38). Biased hypermutations were identified in single-stranded RNA viral genomes during lytic and persistent infections (39).
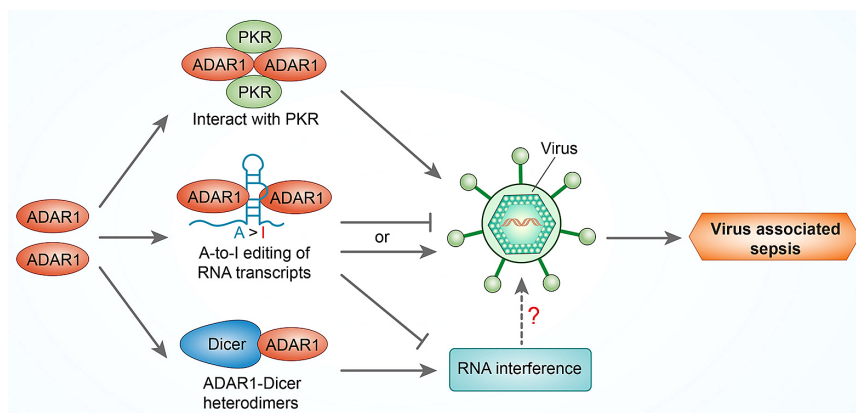
Figure 1. Schematic diagram of the potential relationship between ADAR1 and virus-associated sepsis. ADAR1, an adenosine deaminase, could catalyze A-to-I RNA editing of RNA transcripts. This function enables ADAR1 to cause antiviral and proviral effects by manipulating viral RNAs. In addition, ADAR1 also promotes viral replication by interacting directly with PKR to suppress its kinase activity. Through its A-to-I RNA editing capability and its ability to form ADAR1-Dicer heterodimers, ADAR1 plays important roles in RNAi. However, whether ADAR1 can regulate viral infections via its RNAi function has not been elaborated. Thus, viruses are a key link between ADAR1 and virus-associated sepsis.

In MV infections, the biased hypermutation events are associated with a intensify in viral pathogenesis and persistent infections (40). In addition to these, ADAR-editing of viral genomes is rare and mainly confined to negative-stranded RNA viruses, such as influenza virus A, lymphocytic choriomeningitis virus, respiratory syncytial virus, and paramyxovirus (39–43).

ADAR1 has been shown to inhibit replication of human hepatitis virus like hepatitis delta virus and hepatitis C virus, thereby compromising viral viability (44–46). It is not clear whether this antiviral effect of ADAR1 is a common mechanism of response to viral infections. Paradoxically, ADAR1-L RNA editing actually comprises an essential part of the life cycle of hepatitis D satellite virus (47).

A question has been raised as to why ADAR1 possesses two apparently opposed activities. First, editing of viral RNA does not always culminate in an antiviral effect. When the editing sites are not critical for viral replication, hypermutations of viral RNA may help the virus to escape adaptive antiviral responses (40). This kind of RNA editing plays a proviral role. Thus, RNA editing by ADAR1 could exert an antiviral or proviral effect, depending on the edited sites. Second, the anti- and proviral effects might not spontaneously take place in the same host. The antiviral effect may be observed only when viruses co-localize with ADAR1 and are accessible to dsRNA editing, and when the editing sites are essential for viral replication.

4.2. ADAR1 interacts with protein kinase R to promote viral infections

The IFN-inducible, dsRNA-activated protein kinase R (PKR) is a key dsRNA-binding protein (DRBP) and a serine/threonine kinase. PKR plays a central role in host innate defense strategies, and it possesses pronounced antiviral and antigrowth activities (48–50). Its activation leads to the autophosphorylation and phosphorylation of its downstream targets, including the α subunit of eukaryotic translation initiation factor 2 (eIF2α). Phosphorylated eIF2α prevents translational initiation at viral and cellular mRNAs (50). In addition, its amino-terminus forms complexes with proteins involved in cellular signaling pathways that mediate the activation of the nuclear factor κ B protein complex, which in turn contributes to the production f inflammatory cytokines (51, 52). PKR is extremely effective in restricting the expression and replication of human immunodeficiency virus 1 (HIV-1) *in vitro* (53–55). Nevertheless, HIV-1 replicates efficiently in many cell lines and primary cells, suggesting that the kinase activity of PKR is tightly regulated during natural infections of lymphocytes (56).

As a typical DRBP, ADAR1 contains two putative Z-DNA binding domains and three dsRBDs. Notably, DRBPs are commonly known to stabilize heterotypic protein-protein interactions, and they often mediate down-regulatory functions via PKR during viral infections (33, 57). These observations support the notion that ADAR1 can interact with PKR, inhibit its kinase activity, and suppress eIF2α phosphorylation. Consistent with its inhibitory effect on PKR activation, ADAR1 increases the severity of vesicular stomatitis virus (VSV) infections in $Pkr^{+/+}$ mouse embryonic fibroblasts; however, no significant effect was found in $Pkr^{-/-}$ cells. Such proviral effect of ADAR1 requires its amino-terminal domains but does not require its deaminase domain (8). These findings reveal a novel, proviral role of ADAR1, suggesting that ADAR1 can enhance VSV replication via a mechanism independent of dsRNA editing.

The proviral effect of ADAR1 may be more important when PKR activation is sensitive to viral

infection. In addition, further investigation is needed to address the question why ADAR1 negatively regulates PKR activation. It is well known that cells require PKR to adequately respond to different stresses, in addition to viral infections, and to mediate different forms of stress-induced apoptosis (58, 59). This is in agreement with the anti-apoptotic role of ADAR1 during stresses (21). These observations suggest a potential mechanism whereby ADAR1 negatively regulates the pro-apoptotic effect of PKR. VSV may have taken advantage of this mechanism to establish infections in the host. Recombinant VSV is an effective intranasal vaccine vector for gene therapy (60, 61). Thus, these findings also suggest a possible strategy for optimization of recombinant virus production, and the development of new targets for antiviral therapeutics.

5. VIRUS-ASSOCIATED SEPSIS

Sepsis syndrome (i.e., a systemic inflammatory response associated with infection; ranked by increasing severity as sepsis, severe sepsis, and septic shock) is a common and frequently fatal clinical condition. It represents a major, albeit underappreciated, healthcare problem worldwide. Although the reported incidence of severe sepsis varies, ~750,000 people are annually affected by this condition in the United States (62). Severe sepsis is the number one cause of mortality in non-coronary European intensive care units (63). It is concerning that a 75% increase in the number of patients diagnosed with severe sepsis has been observed over the past two decades. This increase may be partly explained by the improved care of the growing number of individuals surviving into their 70s, 80s, and 90s, and by the associated co-morbidities of the elderly (i.e., cancer and diabetes) (64). Therefore, as the general population continues to age, the incidence of sepsis is projected to significantly increase in the forthcoming years, resulting in, for example, over 1 million cases of severe sepsis in 2020 in the United States alone (62).

Sepsis represents a clinical syndrome defined by the presence of both, infection and an uncontrolled systemic inflammatory response (1, 2). Many factors contribute to this outcome, including specific etiologies, patterns of inflammation, underlying immune dysregulation, and delays in prompt diagnosis and treatment. The mortality in sepsis is attributed to an over-stimulated immune system. This notion is based on studies in animals but does not seem to reflect the clinical picture in human (65, 66). Bacterial infections are a major cause of sepsis, and many experimental studies and clinical observations have focused on the pathogenesis and clinical features of sepsis induced by Gram-negative bacteria and their endotoxins. However, viruses are very common pathogens that cause diseases in children, especially infants and young children. As compared with adults, the children's immune system is still developing, and the immune function is incomplete. Thus, it is apparent that we should also attach importance to virus-induced sepsis.

Influenza is one of the most common causes of viral sepsis in children, and it results in one of the highest rates of hospitalizations and the highest number of deaths (67). Although vaccination may prevent majority of influenza-related severe respiratory infections, low vaccination rates (68), decreased vaccine responses in young children, and periods of poor match between the circulating viruses and the influenza vaccine lead to a continuous healthcare burden (69). Although the parainfluenza virus is usually contained to the upper airway and causes croup, mostly in healthy children, it can cause severe pneumonia in children with compromised immune system or respiratory symptoms (70). This is also true for the adenovirus (71).

Bronchiolitis, a viral infection of the lower respiratory tract, is characterized by lung hyperinflation, increased mucous production, air trapping, and wheezing, and affects 1–2% of infants worldwide (72). Although respiratory syncytial virus is detected in majority of infants that are hospitalized because of bronchiolitis in the developed world (73), very few of these infants die if given supportive care. Human metapneumovirus and rhinovirus are increasingly associated with the hospitalization of infants suffering from bronchiolitis (73). Risk factors of life-threatening bronchiolitis and viral sepsis include premature birth, chronic lung disease, congenital cardiac abnormalities, and primary immunodeficiency (74).

Viral-bacterial co-infection occurs in up to 23% of cases of severe pneumonia, resulting in a high likelihood of respiratory failure and septic shock (75). Viral infections are thought to predispose children to bacterial invasion. For example, methicillin-resistant *Staphylococcus aureus* was recently reported to be associated with the mortality of otherwise healthy children infected with the influenza virus (76). This was especially true during the 2009 influenza pandemic when this fatal co-infection, which caused an unrelenting destruction of the lungs despite the use of appropriate antibiotics, was a strong predictor of mortality (77). Although the mechanism underlying a viral-bacterial co-infection is unclear, one study showed that a subgroup of children at the highest risk of influenza-*S. aureus* co-infection was more likely to experience a "cytokine storm" than children infected with influenza alone (78). Neonates are susceptible to severe viral sepsis caused by herpes simplex virus (HSV), enterovirus, and parechoviruses (79–81), and profoundly immunocompromised children with cancer or HIV infections can develop sepsis upon infection

with HSV, acute cytomegalovirus, adenovirus, or Epstein–Barr virus (82–84). Except for influenza virus infections, older children and adolescents with healthy immune and cardiorespiratory systems are rarely hospitalized for viral sepsis.

6. THE POTENTIAL ROLE OF ADAR1 IN VIRUS-ASSOCIATED SEPSIS

As the susceptibility of infants and toddlers to severe infections particularly caused by viruses,. accompaning sepsis becomes the leading cause of death in infants and children worldwide (3–5). It is essential to think seriously about enfficint solutions about virus-associated sepsis. Focusing on the control of viruial infection, we fully analysis the potential application of ADAR1 in this field (Figure 1). ADAR1 modifies the coding and noncoding sequences of cellular and viral RNAs by A-to-I editing, which can modulate the progress of viral infections. ADAR1 also promotes viral replication by directly interacting with PKR to suppress its kinase activity. Given its A-to-I RNA editing capability and ability to form heterodimers with Dicer, ADAR1 plays important role in RNAi. However, whether ADAR1 regulates viral infections via its RNAi function remains to be fully investigated. Thus, viruses might constitute a key link between ADAR1 and virus-associated sepsis that may be exploited for the development of novel therapeutic targets for the treatment of virus-associated sepsis.

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Abbreviations: ADAR, adenosine deaminase acting on RNA; DRBP, double-stranded RNA binding protein; dsRNA, double-stranded RNA; dsRBD, dsRNA binding domain; eIF2alpha, eukaryotic translation initiation factor 2; HIV-1, human immunodeficiency virus 1; HSV, herpes simplex virus; IFN, interferon; miRNA, micro

RNA; MV, measles virus; pri-miRNA, precursor microRNA; SPE, sclerosing panencephalitis; RISC, RNA-induced silencing complex; PKR, protein kinase R; RNAi, RNA interference; VSV, vesicular stomatitis virus

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