Regulation of innate immune responses by rabies virus

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Abstract
Rabies virus (RABV) is an infectious and neurotropic pathogen that causes rabies and infects humans and almost all warm-blooded animals, posing a great threat to people and public safety. It is well known that innate immunity is the critical first line of host defense against viral infection. It monitors the invading pathogens by recognizing the pathogen-associated molecular patterns and danger-associated molecular patterns through pattern-recognition receptors, leading to the production of type I interferons (IFNα/β), inflammatory cytokines, and chemokines, or the activation of autophagy or apoptosis to inhibit virus replication. In the case of RABV, the innate immune response is usually triggered when the skin or muscle is bitten or scratched. However, RABV has evolved many ways to escape or even hijack innate immune response to complete its own replication and eventually invades the central nervous system (CNS). Once RABV reaches the CNS, it cannot be wiped out by the immune system or any drugs. Therefore, a better understanding of the interplay between RABV and innate immunity is necessary to develop effective strategies to combat its infection. Here, we review the innate immune responses induced by RABV and illustrate the antagonism mechanisms of RABV to provide new insights for the control of rabies.

KEYWORDS
apoptosis, autophagy, infectious disease and host defense, innate immunity and inflammation, rabies virus

1 | INTRODUCTION

Rabies is one of the oldest zoonotic diseases caused by rabies virus (RABV). It has been a global concern because after the symptoms of rabies appear, the mortality rate is close to 100%, and it ultimately leads to death; only a very few cases survive with serious sequelae. Although prevention and control of rabies has greatly improved globally and rabies cases are extremely rare in developed countries and regions,¹² it is still at a high level in many developing countries and less-developed countries, mainly concentrated in Africa and Asia.³⁻¹⁵ According to the World Health Organization report in 2017, about 59,000 people die of rabies every year. Except for vaccination and emergency treatment with a specific antiserum after exposure,¹⁶⁻¹⁸ there are no effective drugs for rabies.¹⁹ The pathogenic mechanism of RABV and the relationship between the virus and host immune response are not thoroughly understood, leading to a lag in the development of therapeutic drugs.

RABV has a wide host range with the ability to infect almost all warm-blooded animals. RABV is an enveloped, nonsegmented, single-stranded, negative-sense RNA virus and is classified in the
genus Lyssavirus of the family Rhabdoviridae based on its bullet-shaped morphology. Similar to other members of the Rhabdoviridae family, the RABV genome is ~12 kb encoding five structural proteins: nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G), and RNA-dependent RNA polymerase protein (L). In particular, N and RNA bind closely to form ribonucleoprotein (RNP) complexes. P and polymerase protein L together with RNP form a helical nucleocapsid, which serves as a functional template for RABV RNA transcription and replication. M is located between N and G proteins and interacts with the endomembrane domain of G protein and RNP to participate in virus budding and RNA replication, respectively. G protein occurs on the surface of the viral particle in the form of trimers, and the recognition and adhesion of cell receptors by its outer membrane region are related to viral entry.

The life cycle of RABV comprises a series of continuous processes, namely attachment, entry, fusion, uncoating, replication, assembly, and release. It is difficult to prevent and control RABV mainly due to its unique neurotropism and immune privileges in the central nervous system (CNS). As known, RABV mainly evades hosts through biting by RABV-infected animals and then triggering the specific bind between G protein and nicotinic acetylcholine receptors on muscle cell surface. This process exposes RABV to the specific bind between G protein and nicotinic acetylcholine receptors through biting by RABV-infected animals and then triggering G protein and RNP to participate in virus budding and RNA replication, respectively. G protein occurs on the surface of the viral particle in the form of trimers, and the recognition and adhesion of cell receptors by its outer membrane region are related to viral entry.

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The interferon response is triggered by the recognition of pathogen-associated molecular patterns (PAMPs) by pattern-recognition receptors (PRRs). The interferon-stimulated genes (ISGs) then activate the interferon signaling pathway, which leads to the induction of antiviral and antiproliferative effects.

### 2 | INTERFERON RESPONSE SUPPRESSED BY RABV

Interferons (IFNs) and interferon-stimulated genes (ISGs) play important roles in antiviral infection. IFNs are highly potent cytokines with antiviral, antitumor, antiproliferative, and immunomodulatory functions. They are grouped into three classes: type I, type II, and type III IFNs, based on evolutionary affinities, receptors, and functional activities. Type I interferon (IFN-I) was identified first and is the most studied type of IFN with the most powerful antiviral function. Type II IFN, IFN-γ, can also be induced by RABV infection and has type I IFN-like antiviral activity, both of which regulate innate immune defense by activating the JAK/STAT1-STAT2 pathway.

During viral infection, the retinoic acid-inducible gene I (RIG-I), a typical cytoplasmic RNA sensor, recognizes the viral 5′-triphosphate ssRNA (single strand RNA) that then activates the inhibitor of 1kB kinase-ε and TANK-binding kinase-1 (TBK-1), inducing phosphorylation of interferon regulatory factor 3 (IRF3) and interferon regulatory factor 7 (IRF7). Then phosphorylated IRF3 and IRF7 translocate to the nucleus and form complexes with other transcriptional activators to induce IFN-α/β production. IFN-γ can be induced by cytokines (mainly IL-12 and IL-18) or following activation of pattern-recognition receptors (PRRs) or antigen receptors during infection or tissue injury. Then IFNα/β and IFN-γ are secreted and bind to the type I interferon receptor (interferon-α/β receptor [IFNAR]) and IFN-γ receptor, which activates the intracellular Janus kinase–signal transducer and activator of transcription (JAK–STAT) pathway, mediating STAT1-3 phosphorylation and its dimerization. Heterodimers of STAT1 and STAT2 bind to interferon regulatory factor 9 to form the interferon-stimulated gene factor 3 (ISGF3) transcriptional complex. ISGF3 translocates from cytoplasm to nucleus and binds to DNA containing the IFN-stimulated response element (ISRE) sequences, thereby driving the expression of large numbers of ISGs and exerting antiviral effects. Elevated IFN-γ expression was found to be a key factor in blood-brain barrier (BBB) permeability enhancement and decreased the expression of tight-junction proteins after infection with the avirulent RABV strain. IFN-γ could induce enhanced BBB permeability through a peroxynitrite (ONOO−)-dependent pathway, which allowed immune effectors to enter the CNS and clear RABV.

Many studies have suggested that the IFN signaling pathway is activated by RABV infection. The viral RNA of RABV can be recognized by RIG-I to induce the production of IFN-α/β. A recent study found that infection by virulent RABV strain can upregulate the expression of ISGs in mice brain, including STAT1, IRF1, IRF7, ISG15, Mx1, OASL2, and PSMB8. Another study found that IIGP1 protein, a specific ISG that was upregulated by RABV infection, can reduce viral replication and pathogenicity of RABV. Meanwhile, a number of studies have shown that RABV has evolved a series of immune escape strategies to reduce IFN secretion and maintain the vitality of infected cells to promote viral replication. Virulent RABV strains inhibit IFN significantly more than avirulent strains, leading to the difference in their pathogenicity.

The RABV P protein, M protein, and N protein are all associated with the inhibition of IFN signaling pathway.

#### 2.1 | P protein

The RABV P protein is a cofactor of RNA-dependent RNA polymerase and participates in the transcription and replication of RABV. Five splicing variants of P protein, comprising one full-length phosphorylated protein and four phosphorylated protein-truncated bodies (P2–P5), were detected simultaneously in different RABV-infected host cells. The full-length P protein is a nucleocytoplasmic shuttle protein possessing an N-terminal nuclear export signal (NES) and a C-terminal nuclear localization signal. P3–S variants are mainly located in the nucleus due to the lack of NES, whereas P2 variant containing the NES domain is mainly located in the cytoplasm. As an innate immune antagonist, RABV P protein exhibits...
virus strain–specific antagonism against IFN signal pathway, mainly in three different ways.

On activation of IFN-α, STAT protein is phosphorylated by JAK and translocated to the nucleus to promote the expression of ISGs. It has been shown that the C-terminal structural domain (amino acids 268–297) of RABV P interacts with tyrosine-phosphorylated STAT (pY-STAT) directly, which retains phosphorylated STAT in the cytoplasm and inhibits the IFN signaling cascade. P2 protein has also been shown to inhibit the IFN signaling cascade via the JAK–STAT signaling pathway. In addition, the full-length P protein and truncated P3 can directly interact with pY-STAT1 in the nucleus via its last 10 amino acids, attenuating the binding ability of ISGF3 and pY-STAT1 to ISG promoters ISRE and GAS, thereby suppressing ISG expression in a dose-dependent manner. P protein also blocks the interaction between STAT and nuclear phosphatases, limiting the recycling of STAT for further participation in the IFN cascade response by inhibiting the dephosphorylation of pY-STAT in the nucleus.

IRF3 is an important transcription factor that induces IFN-α/β expression and is mainly located in the cytoplasm. Brzozka et al and other researchers found that amino acids 176–186 of RABV P protein were able to directly interact with TBK-1 in a dose-dependent manner, inhibiting the phosphorylation of IRF3, which in turn affected IFN expression.

Promyelocytic leukemia (PML) protein can be induced by IFN I and II, localized in both the nucleoplasm and nuclear bodies (NBs). PML-NBs have been shown to play an important role in antiviral defense against DNA viruses and intracytoplasmic replicating RNA viruses. During poliovirus infection, p53 protein, induced directly by IFN-α, can be recruited into PML-NBs and perform its specific antiviral function after being modified by phosphorylation, acetylation, and SUMOylation. After RABV infection, RABV P protein can directly interact with the PML RING domain through its C-terminal domain. The PML RING domain is a cysteine-rich, zinc-binding structural domain that mediates protein interactions and is required for PML-NB assembly and PML SUMOylation. The interaction of P with PML leads to the retention of PML protein in the cytoplasm and hinders the localization of PML in PML-NBs. In addition, the interaction between RABV P3 protein and PML results in the enlargement of PML-NBs and the appearance of dense aggregates, which may lead to the dysfunction of PML-NBs. The RING structural domain of PML has been reported to interact with the N-terminal region of the human foamy virus (HFV) transcriptional activator Tas, thereby interfering with the ability of Tas to bind to viral RNA and activate viral gene expression. Similarly, the overexpression of PML protein also partially inhibited the replication of vesicular stomatitis virus and influenza virus probably by the mechanism similar to that of HFV. It is unclear whether it can affect RABV replication through this mechanism, which needs to be further confirmed.

In summary, the RABV P protein could inhibit the IFN pathway in many ways, and it has been proved that compared with the P protein of virulent RABV strains, the avirulent RABV P protein has a reduced ability to mediate STAT1 nuclear translocation and therefore inhibits IFN signaling less efficiently.

2.2 M protein

The RABV M protein, a determinant of RABV pathogenicity, plays a crucial role in viral assembly/budding during RABV replication. Sonthonnax et al reported that M protein is involved in the disruption of the host innate immune defenses by affecting the NF-κB pathway. After viral infection, transcription factors of the NF-κB pathway are activated and subsequently induce the expression of IFN and other antiviral cytokines. M protein interacts with the RelAp43-p105-ABIN2-TPL2 complex, a member of the NF-κB family, effectively inhibiting the NF-κB pathway and suppressing the expression of IFN-β.

M protein also cooperates with the RABV P protein to inhibit the JAK–STAT pathway. Previous studies have shown that M protein is present in JAK1–protein complexes identified by mass spectrometry. In infected cells, M protein interacts with JAK1 and prevents its phosphorylation, thereby interfering with IFNAR signaling to its downstream target STAT. However, when infected cells are stimulated by IFN, M protein shifts to interact with pSTAT1 and increases the stability of the P and pSTAT1 complex, enhancing the pSTAT1 cytoplasmic retention and thereby inhibiting the JAK–STAT signaling pathway. Although the M protein does not affect STAT1 phosphorylation, it serves to control the expression of ISGs by affecting pSTAT1 cytoplasmic retention and restricting STAT1 binding to the ISRE promoter. In summary, M protein inhibits the activation of the JAK–STAT signaling pathway at different stages in many ways.

2.3 N protein

The N protein, as one of the viral proteins, plays an important role in evading innate immunity and regulating host pathogenicity, thereby helping the virus to replicate and spread efficiently in the brain and CNS. The encapsulation of viral RNA by N protein protects the viral RNA from being recognized by RIG-I, thereby evading the activation of the RIG-I-mediated IRF-3 pathway and inhibiting host defense-related gene expression. Masatanari et al found that N protein of virulent RABV can effectively prevent the activation of RIG-I-mediated IRF-3 pathway, thereby inhibiting the production of IFNs, enhancing the virus pathogenicity and lethality in mice. When N gene was replaced with that of the avirulent strain, especially the key amino acids 273 and 394 were replaced, the expression of host IFN-β and CXCL10 was significantly increased, and the neurological signs were significantly alleviated and mortality was reduced. These findings suggest that the RABV N protein plays an important role in evading the innate immune response.

In conclusion, RABV is able to suppress the host IFN response through multiple pathways and influence the onset of subsequent adaptive immune responses to ensure its successful and efficient
replication in host (Figure 1). However, the specific mechanism by which RABV escapes the innate immune response by affecting the IFN pathway is still not fully understood and needs to be further determined in subsequent studies.

3 | AUTOPHAGY INVOLVED IN THE REPLICATION OF RABV

Autophagy is an intracellular membrane trafficking pathway. During autophagy, cytoplasmic components are sequestered by an isolation membrane, and then the membrane expands to form double-membrane vesicles termed “autophagosomes.” After autophagosome maturation, dysfunctional cytoplasmic substances, misfolded proteins, and damaged organelles are wrapped inside autophagosomes and transported to lysosomes for degradation, which then provide energy, amino acids, fatty acids, and other nutrients for cells. Autophagy is always “on” and occurs at a basal level in most host cells.

Many studies have shown that there is a complex relationship between autophagy and invading viruses. Autophagy can serve dual roles in virus infection with either pro- or antiviral functions depending on the virus and the stage of the viral replication cycle. Most negative-strand RNA viruses, including RABV, can induce autophagy during infection. Meanwhile, autophagy induced by viruses can eliminate intracellular viruses through activating various cellular defense responses, such as transmitting viral genome to endosomal toll-like receptors. In addition, autophagy can induce adaptive immune response and promote the degradation of pathogens in the late stage of infection. In turn, many viruses have evolved different strategies to regulate autophagy to promote self-replication. Some viruses even hijack the autophagosomes, which can form a membrane-bound protective environment, to provide the metabolites and energy for self-replication.

Peng et al for the first time identified that the virulent RABV GD-SH-01 infection can activate the autophagy pathway and induce the formation of autophagosomes in human neuroblastoma cells (SK) and mouse neuroblastoma cells (NA), whereas the attenuated RABV HEP-Flury infection was not able to activate this pathway. Conversely, the recombinant HEP-Flury virus carrying the M gene of GD-SH-01 triggered the conversion of LC3-I to LC3-II in SK and NA cells, suggesting that RABVs with different degrees of virulent induce different types of autophagy in the same cells and M protein of RABV may play an important role in this process. Further study confirmed that after infection with virulent RABV GD-SH-01, complete autophagy was induced in SK cells, whereas incomplete autophagy was induced in NA cells, indicating that RABV can induce different degrees of autophagy in different cells. Another study found that both attenuated RABV HEP-Flury and virulent RABV CVS11 can induce incomplete autophagy by...
inhibiting autophagy flux in vitro (NA cells) and in vivo (mice), which is inconsistent with previous studies. This difference may be due to the differences in experimental systems or virus strains, indicating that induction of autophagy by RABV is virus specific. Recently, one study proved that RABV can infect BV2 cells (murine microglia cells that play a role in immune surveillance and immune clearance in CNS) and induce autophagy in a dose-dependent manner, even though the assembly of RABV viral particles was inhibited in BV2 cells.

The mammalian target of rapamycin (MTOR) is a highly conserved protein kinase that can sense pathogen infection and initiate autophagy. AMPK is crucial to regulate cellular responses to metabolic stresses, and it can activate autophagy by inhibiting its main targets, MTOR or the phosphorylation of RPS6KB. To investigate the autophagy pathways involved in RABV infection, Peng et al detected the expression levels of proteins associated with autophagy and found that GD-SH-01 infection increased the ratio of p-AMPK to AMPK, indicating that the AMPK-MTOR pathway was activated after RABV infection. BECN1, one component of the PI3K complex, not only participates in autophagosome formation but also interacts with different protein complexes regulating the autophagosome–lysosome fusion. Liu et al further found that RABV P protein binds to BECN1 and reduces the expression of CASP2, activates the phosphorylation of AMPK, activates the BECN1-CASP2-AMPK-AKT-MTOR and BECN1-CASP2-AMPK-MAPK pathways, and induces incomplete autophagy, providing a scaffold for the replication of RABV genome. Liu et al observed that RABV P5 protein also binds to the BECN1 ring-like structure by directly interacting with the N terminal of BECN1 and triggers incomplete autophagy through BECN1 signaling pathway. In particular, P5 protein wraps immature autophagic vesicles and prevents the fusion of autophagic vesicles and lysosomes, thus facilitating self-replication (Figure 2).

4 | APOPTOSIS INDUCED BY RABV

Apoptosis is a strictly controlled form of programmed cell death, which plays important roles not only in the homeostatic control of cell growth but also in the process of pathogens invading the host. It is characterized by a series of typical morphological features, such as cell shrinkage, membrane-bound apoptotic bodies, membrane exudation, nuclear condensation, DNA fragmentation, caspase activation, and rapid phagocytosis of adjacent cells. Thus far, two main mechanisms have been proposed to explain the activation of apoptotic pathways, including intrinsic and extrinsic pathways. In short, the intrinsic pathway of apoptosis is initiated by the cell itself in response to damage, whereas the extrinsic pathway is initiated by ligand–receptor interactions that occur at the cell surface.

Apoptosis of infected cells is considered to be a defense mechanism to inhibit viral replication. Therefore, many viruses weaken host immune response and promote viral proliferation by evading, hindering, or destroying apoptosis. Si et al found that ORF3-protein of porcine epidemic diarrhea virus can promote virus proliferation by inhibiting apoptosis caused by virus infection. Conversely, apoptosis during some viral infections is considered to enhance viral replication. Owen et al found that caspase-mediated apoptosis is the main mechanism for Colorado tick fever virus to induce host diseases and enhance viral replication, which provides some new insights into the role of apoptosis in virus replication. Interestingly, some viruses can promote their survival and reproduction by modulating host apoptosis at different stages. Influenza A virus inhibits apoptosis at the early stage of virus replication to help virus particles mature properly, whereas it induces the apoptosis process at the late stage of virus replication, promoting the secretion of progeny virus and its infection to adjacent uninfected cells.

During RABV infection, apoptosis occurs in various types of cells, including RABV-infected cells, bystander cells, and infiltrating T cells. Ubel et al showed that RABV induced more than one kind of apoptosis modes in the CNS through cDNA array analysis and proposed two possible apoptosis mechanisms: one is mediated by caspase-1, IL-1β, FasL receptors, and TNF receptors, and the other is associated with protease-mediated processes such as lysosomal proteases and calcium-dependent neutral proteases. Many studies have shown that G protein may play an important role in inducing apoptosis. Faber et al constructed a recombinant RABV containing two identical G genes by reverse genetic system and found that overexpression of G proteins led to apoptosis and enhanced antiviral immune response. A recent study has shown that G protein-coupled receptor 17 can inhibit RABV replication through BAK-mediated apoptosis, which needs to be further confirmed whether it is related to RABV G protein.

Many studies have shown that RABV strains have large differences in triggering apoptosis depending on their different G protein. Préhaud et al compared the apoptosis induced by pathogenic CVS strain and attenuated ERA strain, respectively, and found that ERA can trigger caspase-dependent apoptosis but CVS cannot. RABV G protein is synthesized in the cytoplasm and finally anchored in the cytoplasmic membrane of infected cells. They further found that G protein of ERA accumulates on the cytoplasmic membrane more than that of CVS and forms a sizable ribbon-like protein structure, which may be an important factor in triggering apoptosis. Préhaud et al found that the RABV ATT strain triggered apoptosis of infected neurons, whereas the virulent VIR strain could protect infected cells from apoptosis. Further studies confirmed that the PDZ-binding site (PDZ-bs) of RABV G is crucial for determining the fate of infected cells. The PDZ domain, as one of the important domains that mediate interactions between proteins, is a spherical structure consisting of 80–100 amino acid residues. Their studies showed that microtubule-associated serine/threonine kinase 2 (MAST2) and protein tyrosine phosphatase, non-receptor type 4 (PTPN4) were identified as key neuronal partners of both pathogenic and attenuated RABV strains, and there was no difference in the interaction between MAST2-PDZ and these two G proteins. However, PTPN4-PDZ specifically interacts with the G protein PDZ-bs of attenuated ATT strains. PDZ-bs of VIR and
ATT G proteins disrupt the normal function of MAST2 and PTPN4 by competing with their endogenous partners for MAST2-PDZ and PTPN4-PDZ. In short, the PDZ-bs of different G proteins interacts with or without PTPN4 to affect the neuronal apoptosis. Moreover, RABV M protein plays an important role in regulating cell apoptosis. Zan et al. found that during RABV infection, apoptosis was initially delayed because RABV M protein blocked the activation of Bax, whereas in the late stage of infection, M protein partially targeted mitochondria and induced mitochondrial apoptosis through caspase-dependent and caspase-independent pathways. Compared with previous studies, RABV may have evolved many new strategies to inhibit or activate apoptosis (Figure 3). Therefore, more studies are still required to further elucidate the mechanism of RABV-induced apoptosis, which will benefit our understanding of the replication mechanism of RABV and suppress viral replication by utilizing apoptotic defense mechanisms.

5 | THE INFLAMMATORY RESPONSE TO RABV INFECTION

Inflammatory response is an important part of innate immune response in resisting viral infection. The host can recognize the invading pathogens by PRRs, specifically recognize pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), and then initiate the innate immune response against the virus and activate the NF-κB, JAK–STAT, and inflammasome pathways that are hierarchically linked to inflammatory signaling pathways and trigger a series of inflammatory reactions. It has been reported that during RABV infection, the expression levels of the important cytokines and the inflammatory response are differently mediated by virus with different virulence. A strong inflammatory response is triggered by the fixed strain and effectively clears the virus from the body, whereas infection with street RABV strains triggers little or no inflammatory response. Moreover, the results of differential gene expression analysis in vitro and in vivo revealed that immune-related genes are upregulated by the fixed virus CVS but downregulated by the street strain PB4. More specifically, the expression of various pro-inflammatory chemokines, especially CXCL1, CXCL12, CCL2, and CCL5, and the phosphorylation level of MAPK and NF-κB are significantly increased, and the degree of pathological and inflammatory response is also intensified. NF-κB pathway is considered as the central regulatory mechanism of the inflammatory process to regulate host innate immune response. It has been found that RABV-attenuated strains could induce the maturation of dendritic cells (DCs), resulting in a strong NF-κB immune response,
whereas street strains could avoid to activate DCs, interfering with the process of presenting viruses to immune T cells to escape inflammatory response. Further mapping study revealed that the ectodomain of G protein from different RABVs determines its ability to activate DCs.

NLRP3 inflammasomes are widely involved in the inflammatory response caused by various viruses. NLRP3 inflammasome can be activated by a variety of PAMPs or DAMPs mainly through two processes. One is that toll-like receptor recognizes the corresponding ligands; activates the NF-κB-mediated signaling pathway; and induces the expression of pro-IL-1β, pro-IL-18, and NLRP3. The other activation process is caused by the stimulation of extracellular ATP, pore-forming toxin, or particulate matter, which leads to the assembly and activation of NLRP3 inflammasome, as well as the processing and modification of downstream pro-inflammatory factors by caspase-1 after activation and eventually leads to their mature secretion. It has been proved that RABV can induce the production and processing of pro-IL-1β by activating NLRP3, ASC, and caspase-1-dependent inflammasome to secrete IL-1β in murine bone marrow-derived dendritic cells. Further study needs to be conducted to confirm the role of NLRP3 inflammasome in response to different virulent RABVs.

During neurotropic virus infection, virus-induced cell death and inflammatory cytokine-mediated immune inflammatory responses are closely related, whereas inflammatory cytokine production is usually associated with programmed cell death, of which pyroptosis is a major pro-inflammatory pathway of cell death. A study found that infected Mφ/macrophage with attenuated strain ERA, but not virulent strain CVS11, could significantly cause cell death, including apoptosis and pyroptosis, by promoting the activation of caspase-1 and caspase-3 and the production of IL-1β and IL-18. In vivo experiments revealed that double knockdown of caspase-1/11, (the triggers of pyroptosis), but not caspase-3 (the marker of apoptosis), exacerbates the disease severity induced by ERA, suggesting that caspase-1/caspase-11-mediated pyroptosis plays an important role in suppressing the disease process caused by avirulent RABV strain (as shown in Figure 4).

6 | MITOCHONDRIAL DYSFUNCTION IN THE PROCESS OF RABV REPLICATION

RABV specifically infects neurons and spreads in the host nervous system through retrograde axonal transport, causing acute encephalomyelitis in humans and animals. Initially, the clinical symptoms of rabies were considered to be caused by massive neuronal cell death, but experimental results showed that only low pathogenic strains could induce neuronal apoptosis. Subsequent studies found that RABV infection reduced intracellular ATP level and changed the redox state of cells. The NADH/NAD+ ratio increased, whereas the basic generation of reactive oxygen species (ROS) was not affected in RABV-infected neurons. However, in the presence of mitochondrial substrates and inhibitors, the ROS generation rate in CVS-infected neurons significantly increased. Therefore, RABV infection may induce mitochondrial dysfunction, leading to ROS generation and oxidative stress. A series of studies further confirmed that the severe clinical manifestations of rabies can be caused by neuronal cell dysfunction, and the fundamental reason may be the interaction between RABV P protein and mitochondrial complex I, resulting in excessive reactive oxygen species and axonal injury. It is partly due to the increased production of nitric oxide (NO) by inducible nitric oxide synthase (iNOS) in neurons and macrophages. Increased NO production by iNOS leads to mitochondrial dysfunction and axonal swelling.
In addition, some researchers found that RABV N, P, and G proteins were present in mitochondria using proteomics analysis. Kammouni et al. found that P protein of RABV street strain and Mokola strain could enhance the activity of mitochondrial complex I and increase the level of ROS. The amino acids 139–172 of P protein could interact with mitochondrial respiratory chain complex I (MRCC-I), and S162 and S166 are the critical sites in P protein for this interaction. This interaction increases the activity of MRCC-I, resulting in the increase in ROS level and mitochondrial dysfunction. Besides, phosphorylated STAT3 can be recognized by P protein, and this heterodimer can regulate mitochondrial function. In addition to P protein, 77 amino acids of M protein can target mitochondrial movement, inhibit cytochrome C oxidase activity, and induce mitochondrial dysfunction. Mitochondrial dysfunction can lead to dysfunction of nerve cells, indicating that some clinical symptoms are caused by nerve cell dysfunction. The study on the mechanism of mitochondrial dysfunction can provide drug targets for the treatment after onset (as shown in Figure 4).

**FIGURE 4** RABV (rabies virus) infection induces inflammatory response and mitochondrial dysfunction. RABV infection of mouse bone marrow–derived dendritic cells (BMDC) induces the production of pro-interleukin-1β (pro-IL-1β), leading to the secretion of active IL-1β through the activation of NLRP3, ASC, and caspase-1 complex-dependent inflammatory vesicles. In addition, caspase-1 and caspase-11, the key effectors of inflammatory response, can be activated by the attenuated RABV strain ERA to trigger pyroptosis. Meanwhile, RABV infection can decrease the intracellular ATP levels, alter the redox status of cells, and increase the NADH/NAD+ ratio. RABV P protein interacts with mitochondrial respiratory chain complex I, leading to elevated levels of reactive oxygen species (ROS) and inducing functional mitochondrial disorders. M protein can also target mitochondrial movement to inhibit cytochrome C oxidase activity and induce mitochondrial dysfunction.

**CONCLUSIONS AND FUTURE DIRECTIONS**

It is well known that the host innate immunity against early infection of pathogens is accomplished by a complex process coordinated at many levels. This paper discussed the relationship between innate immune response and RABV in detail. The host innate immune will be activated by the early infection of RABV, which plays a role in defending and eliminating RABV and modulating the adaptive immune response against RABV. However, under immune response, RABV has evolved a variety of escape strategies in the process of coevolution with hosts, mainly through blocking the host innate immune pathways by viral proteins to inhibit the generation of IFN and inflammatory cytokines, balancing its own survival and the host antiviral response. We reviewed the innate immune responses induced by RABV, including IFN response, autophagy, apoptosis, inflammatory response, and mitochondrial dysfunction. Meanwhile, we also described the key roles of RABV structural proteins P, M, N, and G in the negative regulation of antiviral innate immune response. However, still many mechanisms need to be studied to reveal the escape strategies of RABV against the innate immune system.

Many studies have showed that the posttranslational modifications (PTMs) of proteins have an important impact on the function of proteins. During virus infection, host proteins control the virus replication by exploiting PTMs to activate immune response pathways and then eliminate the virus from the host. Meanwhile, viruses hijack the PTM machinery of hosts to modify viral proteins to achieve their efficient replication and survival. Therefore, the PTMs of RABV-encoding proteins and host factors, such as
acetylation, phosphorylation, ubiquitylation, and SUMOylation, need to be further studied to confirm their influence on the function of viral and host proteins in regulating the innate immune response, increasing the understanding of the replication process and pathogenesis of RABV. Due to the unique neurotropism of RABV and the limitations of in vivo experiments, there is an immediate need to establish proper animal models or organoid to reveal the immune escape mechanism of RABV in the nervous system, which will be an interesting direction in the future.

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AUTHOR CONTRIBUTIONS
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CONFLICT OF INTEREST
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