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Concepts in the pathogenesis of rabies

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Abstract

Rabies is a zoonotic disease that remains an important public health problem worldwide and causes more than 70,000 human deaths each year. The causative agent of rabies is rabies virus (RV), a negative-stranded RNA virus of the rhabdovirus family. Neuroinvasiveness and neurotropism are the main features that define the pathogenesis of rabies. Although RV pathogenicity is a multigenic trait involving several elements of the RV genome, the RV glycoprotein plays a major role in RV pathogenesis by controlling the rate of virus uptake and trans-synaptic virus spread, and by regulating the rate of virus replication. Pathogenic street RV strains differ significantly from tissue culture-adapted RV strains in their neuroinvasiveness. Whereas street RV strains are highly neuroinvasive, most tissue culture-adapted RV strains have either no or only limited ability to invade the CNS from a peripheral site. The high neuroinvasiveness of pathogenic street RVs is, at least in part, due to their ability to evade immune responses and to conserve the structures of neurons. The finding that tissue culture-adapted RV strains replicate very fast and induce strong innate and adaptive immune responses opens new avenues for therapeutic intervention against rabies.

Keywords

antiviral immunity; apoptosis; pathogenicity determinant; rabies virus; virus replication; virus spread; virus uptake

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The biology of rabies virus

Rabies is a CNS disease that is almost invariably fatal. The causative agent is rabies virus (RV), a negative-stranded RNA virus of the rhabdovirus family, which has a relatively simple, modular genome organization and encodes five structural proteins: a RNA-dependent RNA polymerase (L), a nucleoprotein (N), a phosphorylated protein (P), a matrix protein (M) and an external surface glycoprotein (G). The N, P and L proteins form, together with the genomic RNA, the ribonucleoprotein complex (RNP). G is the only RV antigen capable of inducing the production of RV-neutralizing antibodies, which are the major immune effectors against a lethal RV infection. On the other hand, the RNP complex was shown to be the major RV antigen capable of inducing $CD4^+$ T cells that can augment the production of RV-neutralizing antibodies through intrastructural antigen recognition [1]. The RNP may play a significant role in the establishment of immunologic memory and long-lasting immunity [1,2].

RV has a broad host range and can infect almost all mammals. Although there have been several routes of transmission reported for RV, natural infection most frequently occurs via a bite. In addition to bites, consumption of RV-infected carcasses might contribute to RV infection in arctic foxes [3], and contact of RV with mucous membranes was found to be another possible route of transmission [4]. In certain, unusual circumstances such as accidental release of aerosolized RV in the laboratory [5,6] or aerosolized RV in caves that are occupied by large numbers of bats [7], transmission via aerosol can occur.

Whether or not street RVs and mouse-adapted or tissue culture-adapted RV strains replicate at the inoculation site before they invade the CNS is still not clear. While experimental intramuscular infection of young hamsters or raccoons with street RV revealed RV replication in striated muscle cells before the virus invaded the axons of motor neurons through neuromuscular junction [8,9], intramuscular infection of mice with the mouse-adapted CVS-24 RV strain showed that RV migrates directly into the CNS without prior replication at the inoculation site [10]. After entering unmyelinated axon terminals, RV is retrogradely transported to the cell body. Recent findings indicate that hitchhiking axonal vesicle transport may represent a key strategy to move virions over long distances in axons [11]. It has been estimated that RV migrates within the axons at the rate of 3 mm/h [12]. The infection then spreads through a chain of neurons that are connected by synaptic junctions. However, the exact mechanism that facilitates the trans-synaptic spread is still unknown. After infection of the brain, the virus spreads centrifugally to the peripheral and autonomic nervous system in many peripheral organs [13]. In the final stage of the infection cycle, RV migrates to the salivary glands; after replication in mucogenic acinar cells, it is shed into the saliva and is ready to be transmitted to the next host [14].

With respect to RV-induced pathology, apoptotic cell death has been proposed as a potential pathogenic mechanism in experimental rabies models of mice infected with a fixed RV strain [15]. However, infection of mice with the silver-haired bat-associated RV does not result in the induction of apoptosis [16]. Furthermore, RV infection causes only limited gross or histopathological lesions in the brains of human rabies patients despite the severe clinical neurological signs of rabies [13]. Unlike other acute viral infections of the CNS, hemorrhages or tissue necrosis is not commonly observed in the RV-infected brain [15]. The pathogenic mechanism that might contribute to the profound CNS dysfunction, characteristic of rabies, could be the impairment of neuronal functions. It has been shown that expression of housekeeping genes is markedly decreased in RV-infected neurons, resulting in a generalized inhibition of protein synthesis [17], and several studies have shown defective neurotransmission following RV infection. Tsiang demonstrated that the binding of acetylcholine receptor antagonist to infected rat brain homogenates was decreased when compared with controls [18]. RV-infected rat brain also exhibited impairment of both the

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release and binding of serotonin, a neurotransmitter involved in controlling the sleep cycle, pain perception and behavior [19,20]. In addition to the effects on neurotransmission, RV infection might have effects on ion channels as well. Infected mouse neuroblastoma cells show a reduction in functional expression of voltage-dependent sodium channels, which could prevent action potentials and ultimately result in functional impairment [21].

In addition to the lack of major pathological lesions in the CNS, no immune response is detectable in most cases of human rabies at 7–10 days after onset of clinical signs [22]. These profound differences, between the pathogenesis of rabies and that of most other viral or bacterial infections of the CNS, are further evidenced by the fact that immunosuppression either has no effect or is detrimental to the outcome of rabies [23]. The low level of immune response often seen in rabies victims is puzzling since it cannot be explained by a weak immunogenicity of RV antigens. In fact, RV G and the nucleocapsid protein are potent B- and T-cell antigens when administered parenterally [24]. A possible explanation for the low degree of immune responses against RV in human rabies patients or animals might be that RV infection of the CNS induces immune suppression [25] and it has been proposed that RV uses a subversive strategy including the prevention of apoptosis and the destruction of invading T cells [26].

Attenuated RV strains that have been adapted to non-neuronal cells differ significantly from pathogenic street RV strains in their neuroinvasiveness, which refers to their ability to invade the CNS from a peripheral site. In this regard, the tissue culture-adapted RV strains have either no or only limited ability to invade the CNS from a peripheral site, whereas street RV strains or mouse-adapted RV strains such as CVS-24 are highly neuroinvasive [27]. The key factors involved in neuroinvasion by RV are virus uptake, axonal transport, trans-synaptic spread and the rate of virus replication.

Until recently, our knowledge of RV pathogenesis was limited and largely based on descriptive studies with street RV strains or experimental infection with attenuated laboratory-adapted strains. The advent of reverse genetics technology made it possible to identify viral elements that determine the pathogenic phenotype of RV and to obtain a better insight into the mechanisms involved in the pathogenesis of rabies.

Identification of viral elements that control uptake, spread & replication of RV

Viral elements involved in virus uptake

RV infection begins with viral attachment to a putative cellular receptor. Although several membrane surface molecules have been proposed as RV receptors, including nicotinic acetylcholine receptor [28], neural cell adhesion molecule [29] and low-affinity neurotropin receptor $p75^{NTR}$ [30], it is still not clear whether these molecules actually play a role in the life cycle of RV. In this context, it has been recently shown that a RV G–$p75^{NTR}$ interaction is not necessary for RV infection of primary neurons [31]. After receptor binding, RV is internalized via adsorptive or receptor-mediated endocytosis [32,33]. Then, the low pH environment within the endosomal compartment causes a conformational change in RV G, which triggers the fusion of the viral membrane to the endosomal membrane, thereby releasing the RNP into the cytoplasm [34]. On the viral side, RV G plays a crucial role in virus uptake, most likely via the interaction with putative cellular receptors that facilitate fast uptake. In this respect, it has been demonstrated that the pathogenicity of tissue culture-adapted RV strains (e.g., ERA, HEP and CVS-11) correlates with the presence of a determinant located in antigenic site III of the G protein [35]. An Arg→Gln mutation at position 333 in this antigenic site of the ERA G protein resulted in a sevenfold delay of internalization of the Gln333 RV variant as compared with that of the wild-type. An Asn_{194}→Lys_{194} mutation in RV G, which accounted for the re-emergence of the pathogenic phenotype, was associated with a significant decrease in the internalization time [36]. Furthermore, experiments with chimeric RVs revealed

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that the time necessary for internalization of RV virions was significantly increased and the pathogenicity was strongly reduced after exchange of the G gene of the highly pathogenic RV strain SB, which was derived from a cDNA clone obtained from the silver-haired bat-associated RV-18 strain [37], with that of a highly attenuated SN strain, which was rescued from a cDNA clone of the SAD B19 RV vaccine strain [38]. Together, these data support the notion that the kinetics of virus uptake, which is a function of RV G, is a major factor that determines the pathogenicity of RV.

Viral elements involved in virus spread & transport

A unique property of RV is its ability to spread from cell-to-cell. The observation that the Gln333 ERA variant loses pH-dependent cell fusion activity *in vitro* [39,40] and exhibits a strongly reduced ability to spread from cell to cell [39,41,42] suggests that RV G also plays a pivotal role in cell-to-cell spread and thus viral transport, probably through its fusiogenic activity. This possibility is further supported by the finding that the rate of spread of pathogenic RV revertant SPBNGAK is almost twice that determined for the nonpathogenic SPBNGA variant [36]. Interestingly, the $Asn_{194} \rightarrow Lys_{194}$ mutation in the G of SPBNGAK caused a shift in the pH threshold for membrane fusion to a higher pH, supporting the hypothesis that a higher pH threshold for membrane fusion is associated with an increase in virus spread [43].

Transneuronal tracer studies of RV infection in rats [44] and rhesus monkeys [45] showed that RV migrates exclusively in the retrograde direction in axons. Although several RV proteins have been implicated in neuronal transport mechanisms, RV G appears to play a predominant role in the transneuronal spread of a RV infection. For example, while peripheral infection with an equine infectious anemia virus (EIAV) pseudotyped with RV G results in viral transport to the spinal cord, the same EIAV pseudotyped with vesicular stomatitis virus G was unable to invade the nervous system [46]. In addition, the finding that viral spread of the ERA G $Arg_{333} \rightarrow Gln_{333}$ mutant within the CNS is strongly reduced as compared with that of the wild-type [39], also points to the function of intact RV G in trans-synaptic spread. The strongest evidence for an essential role of RV G in trans-synaptic transport, however, comes from intracranial infection of mice with a RV G-deficient recombinant virus, which showed that the infection remained restricted to neurons at the inoculation site without any signs of spread to secondary neurons [47]. However, it is likely that, in addition to RV G, RV M also plays a role in virus spread and thus in trans-synaptic transport. In this respect it was shown that spread of the chimeric RV SN-BMBG variant, which contains both M and G from the highly pathogenic SB, was significantly higher than that of the chimeric SN-BG or SN-BM, which contain G and M from SB, respectively, suggesting that an optimal interaction of M with G might play an important role in virus cell-to-cell spread [48]. Since RV M supports virus budding [49], it is likely that the more efficient spread of the chimeric RV SN-BMBG variant is due to optimal virus budding at the postsynaptic membrane.

Recent studies suggested that an interaction between RV P and the dynein light chain links the RV RNP to the host cell transport system, thereby facilitating retrograde axonal virus transport [50,51]. However, peripheral infection of adult mice showed that removal of the LC8 binding domain from RV P does not prevent virus entry into the CNS suggesting that the RV protein is not directly involved in the retrograde axonal spread of RV [52].

Viral elements that control virus replication

Unlike many other viruses, such as influenza virus, the pathogenicity of RVs correlates inversely with the rate of viral RNA synthesis and the production of infectious virus particles. Comparison of the levels of viral mRNA and genomic RNA produced by different chimeric viruses suggests that viral RNA transcription and replication are regulated by several factors including RV M, which has been identified as a trans-acting factor that mediates the switch

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from initial high levels of mRNA synthesis to genomic RNA synthesis [53]. Furthermore, the M of all rhabdoviruses is able to shut down viral gene expression by binding to the RNP, resulting in a highly condensed skeleton-like structure that is unable to support RNA synthesis [49].

To identify other viral elements that control pathogenicity by regulating virus replication, 5′-end sequences of the highly pathogenic SB strain were exchanged in a stepwise manner with those of the highly attenuated vaccine strain SN, resulting in the recombinant viruses SB2 (trailer sequence [TS] + L), SB3 (TS + L + pseudogene [Ψ]), SB4 (TS + L + Ψ + G) and SB5 (TS + L + Ψ + G + M) [37]. Intramuscular infection with parental viruses SB and SN, and with the chimeric RVs SB2, SB3, SB4 and SB5, revealed the highest mortality rates in mice infected with SB and no morbidity or mortality in mice infected with SN. Replacement of TS, L and Ψ in SB with the corresponding elements from SN resulted in a moderate decrease in morbidity and mortality, and additional exchange of G or G plus M strongly reduced or completely abolished virus pathogenicity. Phenotypic characterization of these wild-type and chimeric RVs in tissue culture revealed that the pathogenicity of a particular RV correlates inversely with its ability to replicate in neuronal cells. While SB replicated at levels almost 1000-times lower than SN, and replacement of TS, L and Ψ in SB with those of SN had little effect on virus growth kinetics, the additional replacement of G or G plus M of SB with the corresponding genes of SN resulted in a 1-log increase in virus production, suggesting that the kinetics of viral RNA replication, as well as virus particle production, are largely controlled by the RV G protein. This conclusion is supported by data obtained with RV G variants that differ in a single amino acid in their G proteins [36]. The pathogenic RV variant $SPBNGAK_{194}$ produced a virus titer in NA cells that was 1-log unit lower than that produced by the nonpathogenic $SPBNGAN_{194}$ variant, and real-time PCR analysis revealed that the rate of viral RNA transcription and replication in NA cells infected with SPBNGAN is five- and ten-times higher, respectively, than in NA cells infected with SPBNGAK [54]. Additional evidence for an inverse correlation between pathogenicity and the rate of viral RNA synthesis and virus particle production was obtained from mice infected with chimeric recombinant viruses in which the G and M genes of the attenuated SN strain were exchanged with those of the highly pathogenic SB strain [48]. These experiments revealed a significant increase in the pathogenicity of the SN parental strain bearing RV G from the pathogenic SB strain. The pathogenicity was further increased when both G and M from SB were introduced into SN. Replacement of G or M or both in SN with the corresponding genes from SB was associated with a significant decrease in the rate of production of virus particles as well as in the rate of viral RNA synthesis. These data indicate that both G and M play an important role in RV pathogenesis by regulating virus replication. The finding that replacement of G or G plus M in SN by the G or G plus M of SB results in a moderate or strong decrease in viral RNA transcription and replication, respectively, while replacement of only the M in SN with the SB M results in a strong increase in viral RNA transcription and replication, indicating that RV G also has an important regulatory function in viral RNA transcription/replication, either alone or via interaction with the M protein. The mechanism by which the RV G gene controls viral RNA synthesis is not known. Certain nucleotide sequences within the RV G genes such as those including the codons for Arg_{333} and Lys_{194} have been identified as targets for cellular miRNAs. It has been shown that target recognition by cellular miRNAs can result in positive or negative regulation of virus replication [55–57]. The substitutions Arg_{333}→Glu_{333} or Lys_{194}→Ser_{194} within the RV G gene sequence result in abolition of the target sequences for miRNAs, which in turn is associated with a significant increase in the rate of viral RNA synthesis [Faber M, Thomas Jefferson University, PA, USA, Unpublished Data] suggesting that host cell miRNAs also play an important role in the regulation of RV replication as it has been shown for other RNA viruses including vesicular stomatitis virus [55] and HCV [56,57].

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The regulation of viral replication appears to be one of the important mechanisms involved in RV pathogenesis. To evade the immune response and to preserve integrity of the neuronal network, pathogenic RV strains, but not attenuated strains, can regulate their growth rate. A lower replication level probably benefits the pathogenic RV strains by conserving the structure of neurons that are used by these viruses to reach the CNS. Another explanation for the lower replication rate of pathogenic RVs is that in order to evade early detection by the host immune system, the virus keeps the expression levels of its antigens at a minimum.

Relationship between RV G expression, apoptosis & pathogenicity

It is well known that street RV strains, which are considerably more pathogenic than tissue culture-adapted strains, express very limited levels of G and do not induce apoptosis until late in the infection cycle, suggesting that the pathogenicity of a particular virus strain correlates inversely with RV G expression and with the capacity to induce apoptosis [58]. Direct evidence for a correlation between the G expression level and extent of apoptosis was obtained with the recombinant RV SPBNGA-GA, which carried two identical G genes and overexpressed RV G [59]. Morphological studies with neuron cultures infected with this recombinant RV showed that cell death increased significantly in parallel with the overexpression of RV G and that apoptosis is the primary mechanism involved in RV G-mediated cell death. In particular, the decrease in F actin staining after infection with SPBNGA-GA is consistent with apoptosis-induced depolymerization of the actin filaments. Furthermore, the number of TUNEL-positive nuclei in SPBNGA-GA-infected neurons increased greatly compared with numbers in uninfected and SPBNGA-infected neurons. However, the mechanism by which the RV G gene mediates the apoptosis signaling process remains largely unknown. It has been speculated that RV G expression exceeding a certain threshold severely perturbs the cell membrane, resulting in the activation of proteins that trigger apoptosis cascades [59]. It is very likely that apoptotic cells are not rapidly cleared in the CNS and therefore undergo secondary necrosis [16,60]. Alternatively, RV infection and, in particular, the overexpression of the RV G protein might result in pyroptosis, a cell death pathway similar to apoptosis, which, in contrast to apoptosis, involves the activation of caspase 1 and thereby leading to necrosis [61]. The extent of necrosis or pyroptosis caused by an RV infection likely playes a decisive role in the induction of antiviral immunity. While apoptotic cells maintain their membrane integrity and do not stimulate innate immune responses, necrotic cells become permeable and release endogenous adjuvants that can trigger a robust innate immune response [62].

Since the level of apoptosis/necrosis correlates with the immunogenicity of RV, it has been hypothesized that the immune-enhancing effect of apoptotic/necrotic cells most likely contributes to the generation of a protective immune response [59]. Consequently, the regulation of the expression of RV G is very likely an essential factor in the pathogenesis of rabies as it provides a means for pathogenic RV variants to survive and spread within the nervous system without causing overt neuronal damage and induce a protective immune response that would interfere with the infection.

The expression of RV G can be regulated at the level of RNA synthesis, the post-translational level, or both. It has been shown that the levels of RV G expressed by different chimeric RV variants are reflected by the rate of viral RNA synthesis, indicating that the differential regulation of RV G expression by these variants is the result of variations in the rates of transcription of viral mRNAs [48]. As is the case with the rate of viral RNA transcription, the amounts of RV G expressed by these variants inversely correlates with virus pathogenicity [48]. On the other hand, infection of primary neuron cultures with the less pathogenic RV variant CVS-B2c resulted in fourfold higher levels of G protein than infection with the highly pathogenic CVS-N2c variant despite the synthesis of comparable G mRNA levels in both infections [58]. Pulse–chase experiments showed that the higher G protein levels in CVS-B2c-

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infected neurons were largely the result of the lower degradation rates of the CVS-B2c G protein compared with CVS-N2c G protein [58]. However, the mechanism that leads to faster proteolytic degradation of the CVS-N2c G protein needs to be elucidated.

The role of expression of host cell factors in RV pathogenesis

The expression level of RV G correlates directly with the extent of neuronal apoptosis, inflammatory lesions in the CNS and anti-RV immunity, but inversely with the pathogenicity of the RV. In this respect it was shown that infection of human monocytes and immature dendritic cells with the nonpathogenic SPBNGAS-GAS resulted in much higher RV G expression than infection with the pathogenic street RV DOG4, which was isolated from the brain of a human rabies victim [63]. Consequently, infection of monocytes and dendritic cells with the nonpathogenic SPBNGAS-GAS induced very high levels of mRNA of genes related to the nuclear factor (NF)-κB signaling pathway including IFN-α mRNA, while infection of these cells with the pathogenic DOG4 RV induced only relative low expression of these genes [64], indicating that infection with the nonpathogenic SPBNGAS-GAS causes a much stronger activation of the NF-κB pathway than infection with the pathogenic DOG4. In turn, the significantly higher expression of NF-κB pathway-related genes, including IFN-α/β, may account, at least in part, for the nonpathogenic phenotype of SPBNGAS-GAS. It is likely that a robust immune response triggered by the strong activation of NF-κB pathway-related genes might serve to confine replication of SPBNGAS-GAS to the primary site of infection and eventually clear the infection, in contrast with the weaker activation of NF-κB pathway-related genes and consequent weaker immune response induced by pathogenic RVs, which might allow the spread of the virus to the CNS [64].

While in most viral infections of the CNS inflammatory reactions in response to viral antigens are central to neuronal damage and correlate with the severity of neurological symptoms, the situation is different in rabies where inflammatory responses in the CNS contribute to virus clearance and survival. For example, mice infected intranasally with a recombinant RV-expressing soluble TNF-α (SPBN-TNF-α^+) exhibited enhanced CNS inflammation, had significantly reduced virus loads in the brain and did not succumb to the infection compared with mice infected with recombinant RV containing an inactivated TNF-α gene (SPBN-TNF-α^-), which only showed minimal inflammation but significantly higher virus loads and a high mortality rate [65].

The induction of the type I interferon (IFN) system is probably the most powerful antiviral response capable of controlling viral infections in the absence of adaptive immunity [66–69]. To evade the action of type I IFNs, many viruses including RV have developed strategies to counteract virus clearance through type I IFNs and viral proteins have been identified that can inhibit IFN-α/β production or action by counteracting IFN gene induction, Jak-Stat signaling and IFN-stimulated gene products. *In vitro* studies have shown that RV P inhibits the production of IFN-α/β through a number of mechanisms, including: abolishing interferon regulatory factor (IRF)-3 phosphorylation, thereby impairing IRF-3 dimerization, nuclear import and transcriptional activity of IRF-3 [70]; binding to Stat1, thereby preventing the nuclear accumulation of phosphorylated Stat1 homodimers or Stat1/Stat2 heterodimer, which plays a crucial role in mediating the activation of the antiviral state [71,72]; and interacting directly with promyelocytic leukemia (PML) protein thereby disrupting the PML nuclear bodies [73]. Whether the interplay between induction of IFN-α/β and countermeasures of RV actually plays a decisive role in *in vivo* RV infection, is somewhat questionable. While IFN receptor$^{-/-}$ mice infected intramuscularly with a non-neuroinvasive RV succumb to the infection, their normal counterparts survive [Dietzschold B, Thomas Jefferson University, PA, USA, Unpublished Data], indicating that IFN-α/β plays an essential role in protection. On the other hand, the finding that mice infected with a highly neuroinvasive RV succumb to the

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infection regardless of whether their IFN signaling pathway is intact or not [Dietzschold B, Unpublished Data], suggests that neuroinvasive RVs may have either the ability to escape from the type I IFN response or do not induce sufficient quantities of IFN-α/β, which are necessary to clear the infection.

Data indicating that the rate of viral RNA synthesis and expression of viral proteins, in particular RV G, correlates inversely with the pathogenicity of a RV [54,58] strongly support the hypothesis that the regulation of viral RNA synthesis and expression of RV G is essential for the life cycle of RV. However, the mechanisms that regulate RV RNA synthesis and RV G expression are not precisely known, nor is it clear how the RV triggers signaling cascades that result in the induction of antiviral responses. Preliminary studies revealed that certain nucleotide sequences (motifs) within the RV G genes are targets for cellular miRNAs and that nucleotide substitutions resulting in the abolition of these target sequences are associated with a significant increase in the rate of viral RNA synthesis, suggesting that host cell miRNAs play an important role in the regulation of RV RNA synthesis (see earlier).

The observation that the expression levels of host genes that are induced during an RV infection correlate with the rate of RV RNA synthesis and with the expression levels of RV G suggests that RV RNA and/or RV G might act as pathogen-associated molecular patterns, which are recognized by particular pattern recognition receptors, such as retinoic acid-induced gene (RIG)-I-like helicases or Toll-like receptors. For example, triphosphate RNA of RV has been shown to specifically activate RIG-I [74]. This possibility is further supported by data demonstrating that the mortality rate following RV infection is strongly increased in IFN receptor$^{-/-}$ mice and in MyD88$^{-/-}$ mice [Dietzschold B, Unpublished Data]. Pathogenic RVs most likely keep the expression of pathogen-associated molecular patterns at a level that is insufficient to trigger pattern recognition receptor-signaling cascades that result in the induction of potent antiviral responses.

Conclusions

Neuroinvasiveness, neurotropism and neurovirulence are the major defining characteristics of RV. The speed of virus uptake, the ability of the virus to spread efficiently from cell-to-cell and the rate of virus replication are the major factors that determine the pathogenicity of a RV. While pathogenicity correlates directly with virus uptake and virus spread, it correlates inversely with the rate of virus replication and the expression level of RV G. The efficient neuronal spread of RV into and within the CNS requires intact structural integrity of the CNS during almost the entire infection cycle. To infect the CNS, pathogenic street RVs have acquired the ability for rapid entry and efficient spread, while producing only low amounts of viral RNA and proteins in order to prevent cell injury.

Although RV neuroinvasiveness is a multigenic trait involving different RV-encoded proteins and transcriptional elements, the major player in the neuroinvasiveness of the virus is RV G. The mechanisms by which RV G mediates neuroinvasion include the facilitation of fast virus entry and fast trans-synaptic spread, and regulation of viral RNA synthesis.

Street RVs and tissue culture-adapted laboratory RV strains differ significantly from each other in their ability to invade the CNS from a peripheral site and to cause lethal encephalitis. Whereas RV strains that were adapted to non-neuronal tissue cultures have either no or only limited ability to invade the CNS from a peripheral site, pathogenic street RV strains are highly neuroinvasive and cause severe neurological symptoms and almost 100% mortality. It is likely that evasion of immunity is, at least in part, responsible for the high neuroinvasiveness of pathogenic street RVs. Pathogenic RVs appear to evade immune recognition through their ability to keep the expression level of their RNAs and antigens, in particular RV G, at a

minimum. By contrast, attenuated RV strains replicate very fast, express large amounts of G protein and induce strong innate and adaptive immune responses that prevent the virus spreading into and within the CNS and finally clear the infection.

Executive summary

Rabies & the biology of rabies virus

- Rabies is an acute encephalomyelitis caused by rabies virus (RV).
- RV can infect almost all mammals, including humans.
- Dogs, wild carnivores and bats are the natural reservoirs of RV.
- RV is a negative-stranded RNA virus that belongs to the rhabdovirus family.
- Neuroinvasiveness, neurotropism and neurovirulence are the major defining characteristics of RV.
- RV is usually transmitted by a bite at a peripheral site, from where, after entering axon terminals, it is transported in a retrograde direction to the CNS.
- Tissue culture-adapted RVs and street RVs differ significantly from each other in their ability to invade the CNS. Whereas tissue culture-adapted RV strains have either no or only limited ability to invade the CNS from a peripheral site, street RV strains are highly neuroinvasive.

Factors that determine the virulence of RV

- The major factors that determine the virulence of RV are virus uptake, cell-to-cell spread, the rate of virus replication and the expression of the RV glycoprotein (G).
- Pathogenicity correlates directly with the kinetics of virus uptake and virus spread, but inversely with the rate of virus replication and the expression level of RV G.

Viral elements that control the pathogenicity of RV

- Pathogenicity of RV is a multigenic trait involving different RV-encoded proteins and transcriptional elements.
- RV G is the major determinant of the pathogenesis of rabies.
- RV G facilitates fast virus entry and fast trans-synaptic spread and regulates the rate of virus replication together with other viral elements.

The role of apoptosis in the pathogenesis of rabies

- The pathogenicity of RV correlates inversely with its capacity to induce neuronal apoptosis.
- There is a direct correlation between RV G expression level and extent of apoptosis.
- In contrast with attenuated RVs, pathogenic street RVs express very limited levels of G protein and do not induce apoptosis or necrosis.

The role of the induction of host cell factors in the pathogenesis of rabies

- Infection with a nonpathogenic RV causes much stronger activation of the nuclear factor (NF)-κB pathway than infection with the pathogenic RV.

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- Strong activation of NF-κB pathway-related genes likely triggers a robust immune response that might serve to confine replication of a nonpathogenic RV to the primary site of infection and eventually clear the infection.
- Weaker activation of NF-κB pathway-related genes and consequently a weaker immune response induced by pathogenic RVs might allow the spread of the virus to the CNS.

Future perspective

Although substantial progress has been made in identifying elements of RV that play a role in the pathogenesis of rabies, it is still unclear which host cell factors are involved in the disease process nor are the mechanisms by which these factors determine the outcome of the disease precisely known. In particular, innate immune recognition and activation in response to RV infection is not well understood and basic studies are needed to delineate the interaction of RV with pattern recognition receptors and subsequent signaling pathways.

The finding that attenuated RV strains replicate very fast and induce strong innate and adaptive immune responses could form the basis for a new postexposure treatment strategy for human rabies. While treatment of RV-infected individuals with a killed RV vaccine is only successful if the vaccine is administered together with antirabies immunoglobulin shortly after exposure, treatment with a live, highly-attenuated RV vaccine that overexpresses RV G could prevent a lethal RV infection even when administered several days after exposure and without the addition of antirabies immunoglobulin. Because the induction of immune effectors capable of aborting an advanced disease process depends on efficient virus replication, only a replication competent live-attenuated RV vaccine is likely to be effective. Therefore, major research efforts should be put into the complete attenuation of these vaccines so that they become absolutely safe, even for severely immunocompromised individuals. The advantage of a live-attenuated recombinant RV vaccine over the currently used killed RV vaccines for postexposure treatment of human rabies would not only rest in its higher efficacy but also in its significantly lower costs since only a single dose of live-attenuated vaccine, compared with multiple doses of a killed RV vaccine, would be necessary. Furthermore, the treatment with a live-attenuated vaccine would make the use of expensive antirabies immunoglobulin dispensable.

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