Original Article

Pathogenicity of Rabies Viruses Isolated in China: Two Fixed Strains and a Street Strain*

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Abstract

Objective To investigate the virulence characteristics of two fixed strains (CTN and aG) and a street strain (HN10) of rabies viruses isolated in China.

Methods ICR mice of different age groups were inoculated with CTN, aG and HN10 rabies virus strains via the intracracerebral (i.c.) or intramuscular (i.m.) routes, and observed for 20 days.

Results The CTN strain was pathogenic to 7-day-old suckling mice that received i.c. inoculations and 3-day-old suckling mice that received i.m. inoculations. The aG strain was pathogenic to 4-week-old mice that received i.c. inoculations and 7-day-old suckling mice that received i.m. inoculations. The HN10 strain was pathogenic to mice of all age groups via both inoculation routes. In moribund mice, the viruses had spread to most regions of the brain. The CTN and HN10 strains had similar dissemination patterns in the brain; both viral antigens could be found in the dentate gyrus (DG), whereas few viral antigens were present in the DG from specimens that had been infected with the aG strain.

Conclusion A comprehensive sequence analysis of the G protein suggested that differences in gene sequences may be responsible for producing strain-specific differences in pathogenicity and distribution in the brain.

Key words: Rabies Virus; Pathogenicity; Distribution; Glycoprotein

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INTRODUCTION

Rabies is a disease of the central nervous system (CNS) that is caused by infection with the rabies virus (RABV) and results in nearly 100% morbidity of infected individuals[1]. These viruses infect their hosts at peripheral sites, enter motor neurons and sensory nerves via neuromuscular junctions, and migrate to the CNS via retrograde axonal transport. At later stages of infection, there is also centrifugal spread to other organs, which can result in extreme exhaustion and a fatal outcome[2-4]. Street strains of RABVs are highly neuroinvasive. Attenuated RABV strains are

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characterized by stable virulence and inoculation periods due to serial passaging through animals or cells, and these strains have a very limited ability to invade the CNS from a peripheral site. Attenuated virus infection triggers a strong, natural and/or acquired immune response that confers protection against the virus[5-6], but some attenuated strains (such as RC-HL) are nonpathogenic in immunocompetent adult mice, even when administered intracerebrally.

Of the five structural proteins encoded by the genome of RABV, the G protein is the only surface protein that not only stimulates the production of virus-neutralizing antibodies[7] but also plays a crucial role in rabies pathogenesis[1]. It is well known that the RABV G protein enables entry into the nervous system from the site of infection by binding to specific neural receptors[8-10], thus contributing almost exclusively to the neurotropism of the virus[1]. The PDZ binding site of the G protein is associated with various cellular partners and determines the capacity of a RABV to promote neuronal survival (a signature of virulence) or death (a marker of attenuation)[11]. Moreover, the G protein also mediates fusion of the viral envelope with endosomal membranes to release the infectious core into the cytosol for viral transcription and replication[12].

In recent years, a large number of RABV strains have been collected and sequenced[13] as part of a national rabies surveillance effort in China. However, the virulence and pathogenic characteristics of these RABVs, which directly impact virus infection, transmission, and distribution in nature, have not been systemically observed and studied. In this study, the pathogenicities of three representative RABV strains isolated in China from different regions at distinct times and from different hosts were investigated.

MATERIALS AND METHODS

Viruses

Three RABVs (CTN, aG, and HN10) were chosen for use in this study because their isolation background, genetic characteristics, and genomic information were known and complete[14-15]. The CTN strain was kindly provided by the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The aG and HN10 strains were obtained from the State Key Laboratory for Infectious Disease Prevention and Control (China). The CTN strain was a laboratory-attenuated virus (GenBank accession no.: EF564174.1) derived from a human isolate that recovered in the Shandong province in 1956 and was subsequently subjected to 56 passages in mice and then passaged 50 times in human diploid cells[16]. The CTN strain has been used as a vaccine strain to produce inactivated rabies vaccines for human use in China since 1983[17]. The aG strain is a fixed virus that was isolated from a rabid dog in Beijing in 1931. It was then passaged 30 times in rabbits and 55 times in primary hamster kidney cells (PHKC) and was then alternately serially passaged in guinea pigs and PHKC. The virus was used to produce hamster kidney cell rabies vaccines for human use in 1981[18]. The full-length genome of aG has been sequenced in our laboratory (unpublished data). The sequence of the G protein was submitted to GenBank (accession no.: AY009097.1). HN10 is a street virus strain that was isolated from a human with rabies in the Hunan province in 2006[15] (GenBank accession no.: EU643590.1).

Animals

ICR mice were selected as the experimental animals and were purchased from the Institute of Laboratory Animal Medicine at the Chinese Academy of Medical Sciences (CAMS & PUMC, China). The ICR mice were divided into six different age groups (1-day-, 3-day-, 7-day-, 2-week-, 3-week-, and 4-week-old) containing 20 mice in each group. Mice were housed in the biosafety level (BSL)-3 facility of the Military Veterinary Research Institute at the Academy of Military Medical Sciences (China). All animal experiments were approved by the Institutional Ethics Committee for Animal Experiments.

Preparation of Virus Stocks

Virus stocks were prepared as previously described[19]. Briefly, 101-day-old suckling mice were inoculated with 10 μL of viral inoculum via the i.c. route. When moribund, the mice were euthanized and their brains were removed under sterile conditions. A 10% (w/v) suspension was prepared by homogenizing the brain in Dulbecco's Modified Eagle medium (Invitrogen, Grand Island, NY, USA). The homogenate was centrifuged at 1500 g for 10 min (at 4 °C) to remove debris, and the supernatant was collected and stored at -80 °C.

Virus Titration and Animal Infection

The titer of the 10% (w/v) viral supernatant was

determined by inoculating the viral inoculum of each strain into 7-day-old ICR suckling mice via the i.c. route, and the 50% mouse i.c. lethal dose ($MICLD_{50}$) was calculated using the Reed-Muench method. The viral inoculums of each virus contained 10^3 $MICLD_{50}$ per 10 μL. The ICR mice (i.c.-infected, *n*=5; i.m.-infected, *n*=5) of each age group were inoculated with 10 μL of each inoculum via the i.c. and i.m. routes, respectively.

Preparation of Frozen Tissue Sections

All mice were anesthetized using 10% chloral hydrate and perfused with buffered 4% paraformaldehyde when they became moribund. Brains were removed and immersion fixed in the same fixative for 24 h at 4 °C. Coronal sections (25 μm) of the cerebrum, the cerebellum, and the brain stem were cut using a Leica CM1900 freezing microtome after immersing the brains in 30% (w/v) sucrose solution for 48 h.

Direct Immunofluorescence

Frozen sections were blocked using blocking solution (0.01 mol/L phosphate buffered saline [PBS] buffer containing 0.3% triton-100, 5% goat serum, and 0.1% tween 20) at room temperature for 1 h, incubated with a fluorescein isothiocyanate (FITC)-labeled rabies virus N protein-specific monoclonal antibody (Millipore, USA) at 37 °C for 30 min, and stained with 4',6-diamidino-2-phenylindole (DAPI) (Dojindo Laboratories, Japan) in the dark at room temperature for 10 min. The sections were next dried at room temperature, mounted using anti-quenching agent, and visualized using an Olympus FV500 confocal microscope (Olympus, Japan). The fluorescence integral optical density (IOD), which represents the expression of each viral antigen, was analyzed using Image Pro-Plus 6.0 software (Media Cybernetics Co., USA). The expression of the viral N antigens was examined in the cerebral cortex, hippocampus, thalamus, hypothalamus, cerebellum, and brainstem.

Crystal Violet Staining

Frozen sections were stained with 0.5% crystal violet for 10 min at room temperature in the dark and were then consecutively immersed in 70%, 75%, and 90% ethanol solution for 30 s each. The sections were then dehydrated in 100% ethanol for 5 min, permeabilized with xylene for 5 min, mounted with resinene, and visualized using a light microscope (Olympus BX41, Japan).

RESULTS

Neuroinvasiveness and Neurovirulence of Three RABV Strains Administered to Mice via I.C. and I.M. Inoculation

ICR mice of six different age groups were infected with the CTN virus strain via i.c. or i.m. inoculation. The suckling ability of the mice in the 1-day, 3-day, and 7-day age groups was observed for 20 days; most animals developed neurological symptoms, such as limb paralysis and hyperspasmia, at 4 days post-i.c. inoculation, and all of the mice died within 2 days of the appearance of symptoms. The mice in the 2-w, 3-w, and 4-w age groups did not show any clinical symptoms at 20 days post-i.c. inoculation. The CTN strain was pathogenic only to mice in the 1-day and 3-day age groups that had received i.m. inoculation. At 5 days post-infection, all of the suckling mice had developed clinical symptoms and died within 2-3 days after the onset of symptoms, whereas mice in the other age groups showed no clinical symptoms post-i.m. inoculation.

The aG strain was lethal to mice of all age groups that had received i.c. inoculations. All of the mice developed neurological symptoms at 4 days post-infection and died within 1-2 days after the onset of symptoms. However, the aG strain administered via the i.m. route was pathogenic only to 1-day-, 3-day-, and 7-day-old suckling mice.

The HN10 strain, when administered via both the i.c. and i.m. routes, was lethal to mice of all age groups. Neurological symptoms appeared at 5 days post-infection, and all of the mice died within 2-3 days after the onset of symptoms.

The results of these animal experiments (Table 1) indicated that infection with the street strain HN10 resulted in a high level of neuroinvasiveness and neurovirulence and was pathogenic to mice of all age groups, even when the animals were infected at peripheral sites. The fixed aG strain displayed a limited ability to invade the CNS from peripheral sites, which led to its pathogenicity only after i.m. administration to suckling mice within 2 w of age. The CTN attenuated strain, when administered via a peripheral site, was lethal only to suckling mice within 3 days of age and was nonpathogenic to mice older than 7 days even when administered via the i.c. route of infection. Therefore, we concluded that the relative neuroinvasiveness and neurovirulence of these three RABVs were as follows: HN10 > aG > CTN.

Table 1. Pathogenicities of the Three RABVs in Mice of Various Age Groups

Age Group	Inoculation Mode	Fatality/Total (Mortality %)		
		CTN	aG	HN10
1 day	i.c.	5 / 5 (100)	5 / 5 (100)	5 / 5 (100)
	i.m.	5 / 5 (100)	5 / 5 (100)	5 / 5 (100)
3 day	i.c.	5 / 5 (100)	5 / 5 (100)	5 / 5 (100)
	i.m.	5 / 5 (100)	5 / 5 (100)	5 / 5 (100)
7 day	i.c.	5 / 5 (100)	5 / 5 (100)	5 / 5 (100)
	i.m.	0 / 5 (0)	5 / 5 (100)	5 / 5 (100)
2 week	i.c.	0 / 5 (0)	5 / 5 (100)	5 / 5 (100)
	i.m.	0 / 5 (0)	0 / 5 (0)	5 / 5 (100)
3 week	i.c.	0 / 5 (0)	5 / 5 (100)	5 / 5 (100)
	i.m.	0 / 5 (0)	0 / 5 (0)	5 / 5 (100)
4 week	i.c.	0 / 5 (0)	5 / 5 (100)	5 / 5 (100)
	i.m.	0 / 5 (0)	0 / 5 (0)	5 / 5 (100)

Note. Intracerebral (i.c.); intramuscular (i.m.).

Distribution of RABV Antigen in the Brain

The experimental infection of animals with RABV at peripheral body sites likely offers the greatest level of insight into the natural distribution of these viruses in the brain. Therefore, the brains of moribund mice infected with these three RABVs were used to observe the distribution patterns of each virus. In 1-day-old moribund mice from each group, the N protein antigen of the CTN strain had spread to almost all areas of the brain (cerebral cortex, hippocampus, thalamus, hypothalamus, cerebellum, and brain stem) and was distributed predominantly in the cerebellum and brain stem. In the hippocampus, antigens were mainly located in the CA1 region and the DG, whereas fewer antigens were localized in the CA3 region (Figure 1A). Antigens from the aG strain were observed in almost all areas of the brain except for the DG region and were primarily located in the cerebellum and brainstem. In the hippocampus, aG antigens were primarily distributed in the CA1 and CA3 regions, and few antigens were observed in the DG (Figure 1B). Although HN10 antigens were widely distributed throughout all parts of the brain, the expression of these antigens was similar for each area of the hippocampus (CA1, CA3, and DG) (Figure 1C).

In summary, antigens from the three RABVs were observed in most areas of the brain, but were predominantly distributed in the cerebellum and brainstem; however, different distribution patterns for the three strains were found in the hippocampus. The HN10 antigens were evenly distributed across each area of the brain, which indicated that HN10 is highly neuroinvasive. In moribund mice, the CTN antigen was identified in each region of the brain, and its distribution pattern was similar to that of HN10, although it was an attenuated virus. Moreover, aG antigens were observed in most areas of the brain, except for the DG region.

In the moribund mice of other age groups, the distribution patterns of viral antigen for each strain were similar to those of mice in the 1-day age group (data not shown), which indicated that the distribution pattern of RABV antigen in the brain following i.m. inoculation was related to the strain of virus but not to the age of the experimental animals.

Expression of Viral N Antigen for Each Strain in the Brain

The IOD analyses for the expression of each of the antigens in the brain are shown in Figure 2. In the hippocampus, the IOD values for the CA1, CA3, and DG regions were calculated. In the thalamus, the following nuclei were involved in the calculations of the IOD: the medial habenular nucleus (MHb), the paraventricular thalamic nucleus (PV), the posterior thalamic nucleus (Po), the ventral posterior nucleus (VP), the mediodorsal the thalamic nucleus (MD), the laterodorsal thalamic nucleus (LD), the ventrolateral nucleus (VL), and the ventromedial thalamic nucleus (VM). In the hypothalamus, the following nuclei were examined for the IOD analysis: the dorsomedial hypothalamic nucleus (DM), and the ventromedial hypothalamic nucleus (VMH).

In the cerebellum, the medial cerebella nucleus (Med), the interposed cerebellar nucleus, the posterior part (IntP), and the inferior cerebellar peduncle (icp) were assessed for the IOD calculation. In the brainstem, the following nuclei were involved in the IOD calculation: the medial vestibular nucleus (MVe), the spinal vestibular nucleus (SpVe), the nucleus of the solitary tract (Sol), the parvicellular reticular nucleus (PCRt), the intermediate reticular nucleus (IRt), the gigantocellular reticular nucleus (Gi), the dorsal paragiantocellular nucleus (DpGi), and the prepositus nucleus (Pr).

The CTN antigen was detected in most brain areas examined. The highest expression level of the CTN antigen was detected in the icp, followed by the

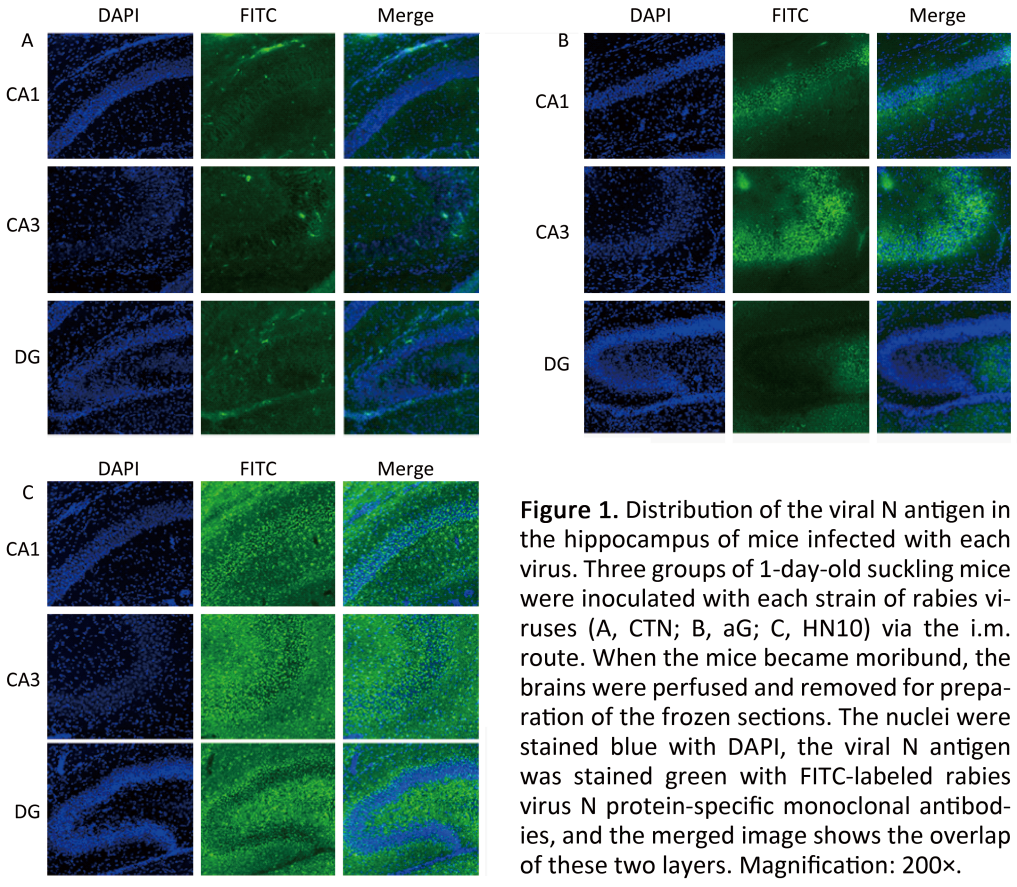

Figure 1. Distribution of the viral N antigen in the hippocampus of mice infected with each virus. Three groups of 1-day-old suckling mice were inoculated with each strain of rabies viruses (A, CTN; B, aG; C, HN10) via the i.m. route. When the mice became moribund, the brains were perfused and removed for preparation of the frozen sections. The nuclei were stained blue with DAPI, the viral N antigen was stained green with FITC-labeled rabies virus N protein-specific monoclonal antibodies, and the merged image shows the overlap of these two layers. Magnification: 200×.

brain stem, the thalamus, the cerebral cortex, and the hypothalamus. The lowest level of expression was found in the CA3 region of the hippocampus (Figure 2A). The aG antigen was observed in all areas of the brain except for the DG, and the expression of this viral antigen was highest in the brainstem and lowest in the DG, where few aG antigens were detected (Figure 2B). HN10 antigens were found in all areas of the brain and showed a high level of expression of the viral N antigen (Figure 2C).

In each area of the hippocampus, the expression of the viral N antigen by each viral strain was significantly different. The expression of the CTN antigen in the CA3 region was significantly lower than that in the CA1 region and the DG ($P_{(CA3,CA1)}$<0.01; $P_{(CA3,DG)}$<0.01) (Figure 2A), and the expression of the aG antigen was significantly higher in the CA3 region than the CA1 region (P<0.01), but a low antigen expression level was observed in the DG ($P_{(DG,CA1)}$<0.001; $P_{(DG,CA3)}$<0.001) (Figure 2B). The HN10 antigen was highly expressed in the CA1 and CA3 regions and the DG (Figure 2C).

Relationship between Hippocampal Neural Loss and Viral Antigen Expression

Because of the observed differences in the dissemination pattern of each viral antigen in the hippocampus, we speculated that a relationship between neural abnormalities and the expression of each viral antigen may exist. Therefore, crystal violet staining was used to observe the morphology of the hippocampal neurons. The staining revealed various extents of neural loss in the CA1 and CA3 regions and the DG in mice infected with the CTN strain. The hippocampal pyramidal neurons in the CA3 region exhibited the most severe deficits, consisting of irregular condensation of the nuclear chromatin, neuropil vacuolation, and neuronal loss. The granular neurons in the DG displayed less irregular nuclear chromatin condensation compared with those in the CA1 and CA3 regions (Figure 3A, B, & C). A large amount of pyramidal neural loss in the CA3 region affected virus survival and replication, and this may have led to the reduced expression of viral antigens observed in these regions.

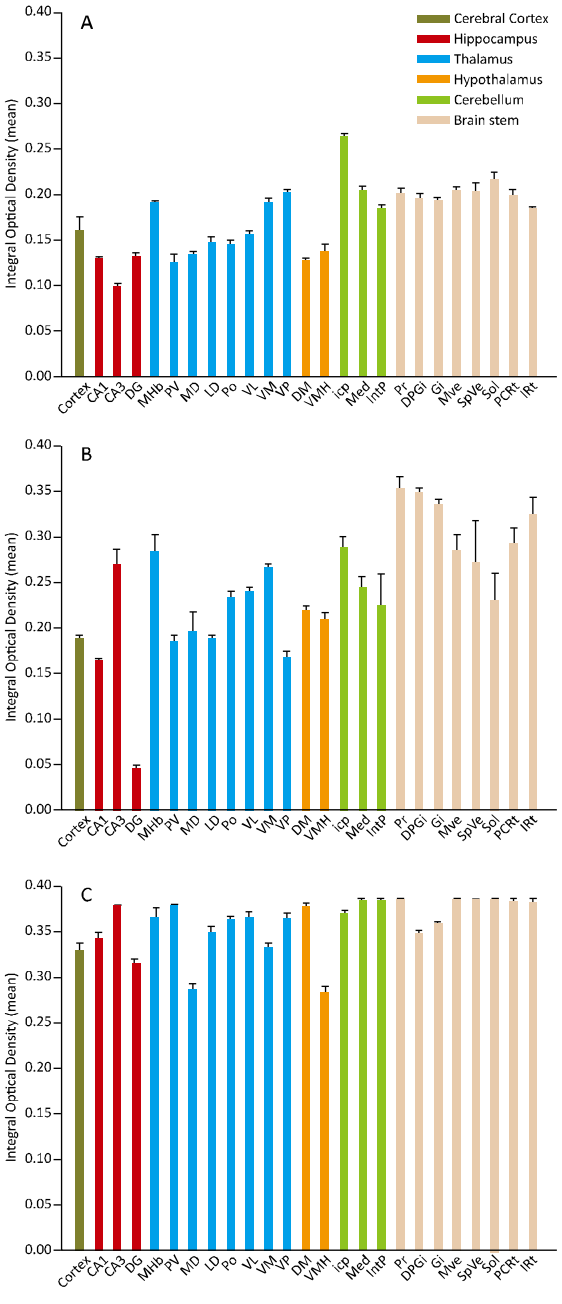

Figure 2. Fluorescence intensity values of each brain region from mice infected with the three viruses. One-day-old suckling mice were infected with each virus via i.m. inoculation, and the IOD for each region was determined when the mice were moribund. A. CTN strain. B. aG strain. C. HN10 strain. The IOD (mean) value represents the ratio of the total fluorescence density to the emitting area. The various colors represent different parts of the brain, such as the cerebral cortex (yellow), hippocampus (white), thalamus (blue), hypothalamus (orange), cerebellum (green), and brain stem (gray).

In the hippocampus of animals infected with the aG strain, neural loss was severe in the CA1 and CA3 regions, consisting of an almost complete loss of pyramidal neurons. In the DG region, irregular nuclear chromatin condensation and neuropil vacuolation were observed in many neurons in the inner layer, although the extent of neural loss was much less than that observed in the CA1 and CA3 regions (Figure 3D, E, & F). Therefore, neural loss was not the only cause of the reduced expression of aG antigens in the DG region, and additional, unknown factors related to tissue tropism need to be studied further.

In the hippocampus of animals infected with the HN10 strain, the pyramidal and granule neural nuclear chromatin exhibited regular condensation, and no neural loss was observed (Figure 3G, H, & I), which exhibited the same morphological feature with that in mock-infected brain (Figure 3 J, K, &L).

DISCUSSION

Different Pathogenicities for Three RABVs

Neuroinvasiveness, neurotropism, and neurovirulence are the major defining characteristics of RABV infections. 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 viral factors that determine the pathogenicity of RABV. Although RABV neuroinvasiveness is a multigenic trait involving different structural proteins and transcriptional elements, the specific viral glycoprotein has the greatest impact on neuroinvasiveness[20-22]. Sequence analyses of the glycoproteins of the three examined RABVs revealed that the CTN and HN10 strains shared the highest degree of amino acid homology (97%), which was much higher than the homology shown between the HN10 and aG strains (90.7%) and that between the CTN and aG strains (90.1%).

The amino acid arginine (Arg or R) at position 333 of the mature G protein (G333) of RABV is a well-defined determinant of virulence in adult mice. If R is replaced by glutamine (Q), leucine (L), or glycine (G), viral virulence is greatly reduced[23-24]. The observation that the CTN RABV strain contains a Q mutation at G333 partially explains its lack of pathogenicity in mice older than 7 days, even when the virus is administered via i.c. inoculation. Some RABVs that contain R at G333 do not cause lethal infection in adult mice (such as RC-HL), but such substitutions have only been identified in fixed strains[25]. In this study, aG was a fixed strain that

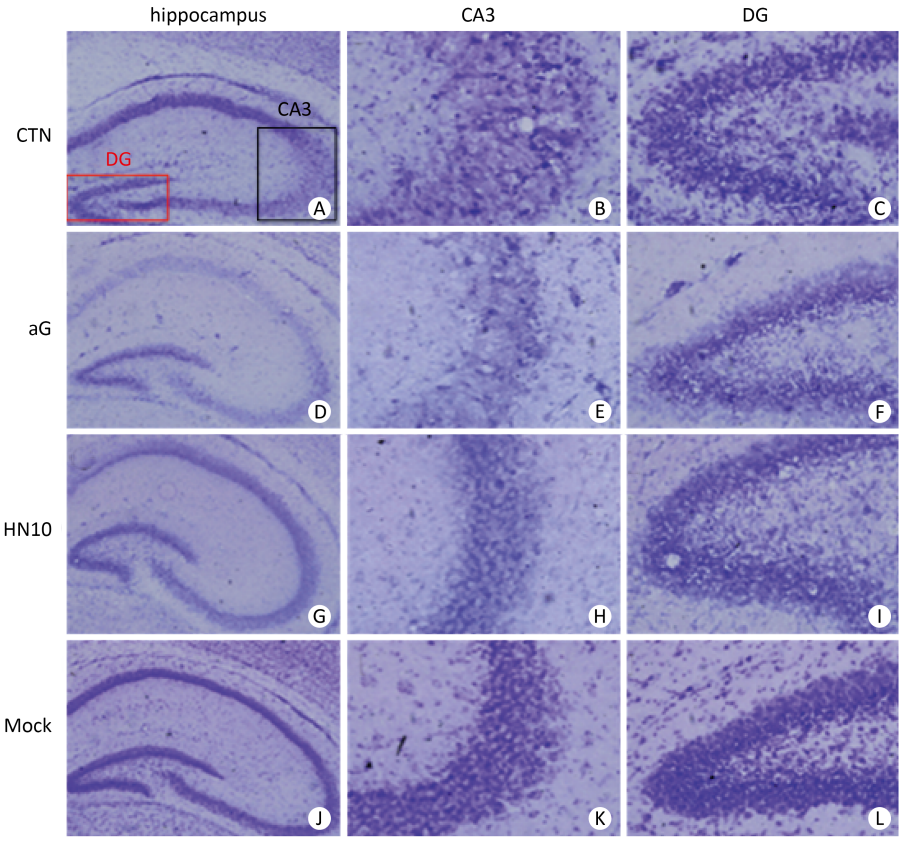

Figure 3. Crystal violet staining of the hippocampus of mice infected with each virus. Three groups of 1-day-old suckling mice were inoculated with each virus via the i.m. route. When the mice became moribund, brains were collected, and frozen sections were prepared. A-C, D-F, and G-I show staining of the entire hippocampus, the CA3 region, and the dentate gyrus (DG) from mice infected with the CTN, aG, and HN10 virus strains, respectively. J-L shows the mock-infected controls. Magnification: A, D, G, J (40×); B, C, E, F, H, I, K, L (400×).

contains R at G333 and was more virulent than the CTN strain.

The G protein of RABV plays an important role in regulating viral replication[26]. The rates of viral RNA synthesis and virus particle production were inversely correlated with pathogenicity. Certain amino acids sequences in the G protein, including R at G333, have been identified as targets of cellular miRNAs. It has been shown that target recognition by cellular miRNAs can result in positive or negative regulation of virus replication[27-29]. The R → Q substitution within the G protein (G333) results in the loss of a miRNA target sequence, which in turn is associated with a significant increase in the rate of viral RNA synthesis. In this study, the aG strain, which is a laboratory-fixed virus, had been subjected to passage in animals and cell cultures, which indicated that the rate of the viral RNA synthesis was much higher than that of its parental strain. Therefore, this may partially explain the significant difference in virulence that was found between the aG and HN10 strains, although the amino acid R was present at G333 in both strains. However, the sequencing analyses of the antigen site III of the G protein (330-338) revealed that the substitution of N336D was present in the CTN strain but not in the aG and HN10 strains. This type of substitution has not yet been identified as a contributor to the reduced pathogenicity of the CTN strain.

The glycosylation sites of the G protein affect its ability to fold into the correct conformation, which is another factor affecting the virulence of the virus. The extracellular domains of the G protein contain varying numbers of glycosylation sites, the basic amino acid sequences are represented by N-X-S/T. The analysis of glycosylation sites for the three

RABVs revealed that three identical glycosylation sites were present in their extracellular domains (1-439); these sites included 37 (NLS), 247 (NLS), and 319 (NKT), whereas another glycosylation site in 21 (NLS) was identified only in the G protein of CTN. The presence of this extra glycosylation site may affect the G protein structure of the CTN strain. In addition, the absence of glycosylation sites in the aG and HN10 strains may enable the complete exposure of important antigenic sites that increase the virulence of these strains[30].

RABV Distribution in the Brain

It has been demonstrated that the G protein is important for the distribution of RABV throughout the CNS. Infection with G protein-deficient RABV caused an infection that was restricted to the primary infected neurons, and these virus particles could not spread to secondary neurons[31]. It has also been shown that the G protein determines the distribution pattern of RABV in the brain[32] and that the substitution of various amino acids, such as 242, 255, and 268, can affect the spread of the virus and its distribution in the brain[33]. Moreover, the L protein appears to be closely associated with pathogenicity, as it has been shown to stabilize the G protein[34]. In our study, the G and L amino acid sequence homology between the CTN and HN10 strains (97% and 98.4%, respectively) was greater than that between the CTN and aG strains (90.1% and 94.5%, respectively) and the HN10 and aG strains (90.7% and 94.4%, respectively). The G proteins of the CTN and HN10 strains contained identical amino acids at the 242 (CTN: G, HN10: A), 255 (CTN: G, HN10: D), and 268 (CTN: G, HN10: I) positions, whereas the aG strain contained a D→G mutation at 255 position. All of the above factors may be associated with the similarity between the brain distribution characteristics of the CTN and HN10 strains. For these two strains, our results indicated that both antigens were observed in the DG region, although fewer aG antigens were observed in the DG region. It has also been demonstrated that different inoculating doses of RABV can affect the proliferation rate of the virus but not the distribution of the virus in the CNS[35-36].

The Innate Immune Response Induced by the Fixed and Street Strains

Fixed and attenuated strains can induce apoptosis, but they do not reach the brains of immunocompetent adult mice after intramuscular inoculation because they instead trigger the premature destruction of the infected neurons, which impedes the neuronal network that allows transmission from one neuron to the next[37-38]. However, the infection of suckling mice, which do not possess a mature immune system, results in the ability of the virus to enter the CNS from a peripheral site and triggers robust innate immune responses in the brain. The link between the activation of an innate immune response and the induction of apoptosis remains unclear, as RABV has been described as capable of initiating TRAIL-mediated[39] as well as mitochondrial-mediated apoptosis[9].

More recently, studies of the M protein of RABV[40] have revealed its role in the virus life cycle and have indicated that, similar to the G protein[41], it contains pro-apoptotic motifs. RABVs typically trigger a robust innate immune response in neurons, although virulent strains do not induce apoptosis, and this virulence is inversely correlated with apoptosis[42]. It has been proposed that pathogenic RABV strains trigger a reduced innate immune response compared with attenuated RABV strains[19] and that pathogenic strains escape innate immune attacks more effectively than attenuated strains. Nevertheless, innate immune response stimulation and IFN production is required to stimulate the immunoevasive strategies of the virus by inducing the upregulation of proteins such as B7-H1 that can kill host T cells[43-44].

In this study, the pathogenic characteristics and viral distribution of three representative Chinese RABVs were systematically investigated, which provided the basis to study the pathogenic mechanisms of these three RABVs and would give more insights into the pathogenic features of Chinese RABVs.

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