Neurotropism and neuropathological effects of selected rhabdoviruses on intranasally-infected newborn mice

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Abstract

Viral neurotropism is the ability of viruses to infect neuronal cells. This is well studied for herpesviruses, rabies-related viruses, and a few others, but it is poorly investigated among almost all arboviruses. In this study, we describe both the neurotropism and the neuropathological effects of Amazonian rhabdoviruses on the brains of experimentally infected-newborn mice. Suckling mice were intranasally infected with $10^{-4}$ to $10^{-8}$ LD$_{50}$ of viruses. Animals were anaesthetized and perfused after they had become sick. Immunohistochemistry using specific anti-virus and anti-active caspase three antibodies was performed. All infected animals developed fatal encephalitis. Survival time ranged from 18 h to 15 days. Viruses presented distinct species-dependant neurotropism for CNS regions. Histopathological analysis revealed variable degrees of necrosis and apoptosis in different brain regions. These results showed that viruses belonging to the Rhabdoviridae family possess distinct tropism for CNS structures and induce different pattern of cell death depending on the CNS region.

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1. Introduction

Infectious diseases are the second leading cause of death in the world and the continual evolution of emerg- ing and re-emerging diseases, particularly in developing countries has heightened their global impact this cen- tury (Fauci, 2001; Whitley and Gnann, 2002). The high incidence of arboviruses in developing countries like Brazil, where more than 500,000 cases are annually reported, is significant (Fauci, 2001). In the Brazilian Amazon, this upsurge has been associated with long term inadequate management of natural ecosystems, con- tributing to the emergence and re-emergence of several arboviruses, some of them becoming important regional and national public health problems (Vasconcelos et al., 1991, 1997, 2001). A total of 190 different arboviruses have been identified as infecting humans, hematophagous insects, and wild and sentinel vertebrates in Brazilian Amazon, including 32 identified as human pathogens.
To date, little information is available on a great number of these viruses except for some basic data such as method of isolation and capacity to infect laboratory animals, and the ability to produce an acute rather than a chronic infection. (Vasconcelos et al., 1991, Verlinde, 1968; Whitley and Gnann, 2002).

Among the isolated viral species, 15 belong to the Rhabdoviridae family. Five genera have been described in this viral family, and two of them have furnished viruses in Amazon: genus Vesiculovirus, which includes the vesicular stomatitis serogroup (VSV), and the Lyssavirus genus containing the rabies and rabies-related viruses (Vasconcelos et al., 2001). Other serogroups also considered to belong to the Rhabdoviridae family (i.e., Hart Park, Kwatta, Timbo and Mossuril) are not assigned to any genera. There are also several other ungrouped and unclassified viruses in the family (Vasconcelos et al., 2001). Information on the isolation of Brazilian rhabdoviruses, summarized in Table 1, has been published elsewhere (Karabatsos, 1985; Travassos da Rosa et al., 1984a,b, 1998).

Viruses from several families can infect the central nervous system (CNS) and produce distinct pathological outcomes (Card and Enquist, 1995; Card et al., 1999; Everett and McFadden, 1999, 2001; Fazakerley, 2004). The pathogenesis of viral invasion of the CNS involves many distinct steps, including replication at the primary site of infection, entry and spread within the CNS, replication in neural tissue, host immune response, and tissue injury (Crotty et al., 2002; Fazakerley, 2002, 2004; Rempel et al., 2004). The strategies used by these viruses to get into the CNS can be both by intraneuronal and/or vascular routes. In the nasal cavity, viruses usually infect the respiratory epithelium and propagate to the CNS via four neuronal pathways: the trigeminal, sympathetic, parasympathetic, and the olfactory routes. The olfactory route is more frequent in subjects with longer survival times (Babic et al., 1994). Once in the CNS, the ability of a virus to selectively infect a given subset of cells relies on a variety of viral and host determinants (Whitley and Gnann, 2002).

There have been many studies dedicated to the neuropathology of experimental viral encephalitis (Chaudhuri and Kennedy, 2002; Fazakerley, 2004; Hinson and Tyor, 2001; Rempel et al., 2004; Sonmez et al., 2004; Whitley and Gnann, 2002). Nevertheless, few studies have investigated the experimental neuropathology of arboviruses (Dobler, 1996; Griffin, 1995; Whitley and Gnann, 2002), and virtually no studies have addressed the neuropathology of emerging Amazonian rhabdoviruses.
In this paper, newborn mice were infected with seven Amazonian rhabdoviruses in order to investigate the neurtropism of these arboviruses at the time they present severe symptoms and associated histopathological outcomes following experimental infection.

2. Materials and methods

2.1. Experimental animals and arboviruses used

Eight-four 2-day old suckling mice (Mus musculus) were infected by intranasal instillation with five different rhabdoviruses belonging to genus Vesiculovirus (Cara-jas, Cocal, Jurona, Maraba, and Piry) and two unassigned viruses (Curionopolis and Iatacialinaus). Another set of animals were used as uninfected control and observed under the same conditions of infected ones. The viral strains were characterized as Rhabdoviridae based on their biological, antigenic and genetic characteristics, complement-fixation and neutralization tests according to protocols described elsewhere (Beaty et al., 1989; Travassos da Rosa et al., 1983, 1984a,b, 2001).

2.2. Experimental viral infection protocol

The study protocol was approved and followed the guidelines of the ethical committee of Universidade Federal do Pará. All procedure steps were performed under negative pressure in a class II B II biosafety cabinet. Viruses-containing brain homogenates were obtained as follows: 0.1 ml of each viral suspension was first inoculated intracerebrally (IC) into twelve newborn mice. After inoculation, the mice were observed daily and when the animals present clinical signs, they were sacrificed and their brains were removed, immediately frozen in liquid nitrogen and stored at −70 °C. Later, brain tissue (0.2 g) was macerated and mixed with 0.8 ml of phosphate borate saline buffer solution (PBS) containing penicillin (100 U/ml) and streptomycin (100 μg/ml). Suspension was clarified by centrifugation at 8000 × g for 15 min at 4 °C. Supernatants were inoculated intranasally (5 μl of each virus suspension into both newborn mouse nostrils using a 10-μl micropipette). The infecting dose of each virus is shown in details in Table 1. After inoculation, the animals were observed daily. When they presented clinical signs or died the survivors were perfused as follow.

2.3. Perfusión and microtomy

Animals were anaesthetized by hypothermia and perfused intra-cardially through the left venticle with 0.9% saline and 4% paraformaldehyde in 0.1 M PBS. After craniotomy, the brains were post-fixed in the primary fixative solution for 24 h and cryoprotected in 25% sucrose/glycerol for 30 min, 50% sucrose/glycerol for about 6 h and 100% sucrose/glycerol overnight. After cryoprotection, the brains were embedded in Tissue Tek gel and frozen at −55 °C using a cryostat with Peltier effect (Zeiss, Germany). Blocks were either further sectioned both coronally and parasagitally at 20 μm thick or kept in freezer at −20 °C for further processing. Sections were directly mounted on gelatin-coated slides during sectioning. air dried for about 24 h, and kept in freezer at −20 °C until histological procedures.

2.4. Histopathological analysis

For histopathological analysis, 10-μm sections were stained by either hematoxylin and eosin or by Nissl technique (cresyyl violet staining). Histopathological evaluation included the search for possible necrotic areas, pyknosis, oedema, and other histopathological signs of experimental infection.

2.5. Antibodies used and immunohistochemical protocol

In order to assess the distribution of the different rhabdoviruses in newborn mouse brain, immunohistochemistry (IHC) was performed on all brains. Specific antibodies against each virus species were produced by Instituto Evandro Chagas following protocol published elsewhere (Beaty et al., 1989).

Slide-mounted sections were removed from freezer, incubated in oven at 37 °C for 30 min, and rinsed once in 0.1 M PBS for 5 min. In order to improve labeling intensity, sections were pre-treated in 0.2 M boric acid (pH 9.0) previously heated to 65 °C for 25 min. This temperature was maintained constant during the pre-treatment period. Sections were allowed to cool for 20 min and incubated under constant agitation in a 1%-hydrogen peroxide solution in methanol for 20 min. Sections were then rinsed in a 0.05% PBS/Tween (Sigma, USA) solution for 3 min (3 times) and incubated with 10% normal horse serum in PBS for 30 min. Sections were then incubated with the primary antibody diluted at 1:10 in 10% normal horse serum for 2 h, rinsed in PBS/Tween solution for 3 min (3 times), and incubated with the biotinylated horse anti-mouse secondary antibody (Vector Laboratories, USA) diluted at 1:100 in PBS for 1 h. As a negative control, normal horse serum rather than the primary antibody was added on some slides for each
antibody used for each virus. Sections were rinsed again for 3 min (3 times) and incubated in the complex avidin-biotin-peroxidase (ABC Kit, Vector Laboratories, USA) according to the protocol previously published (Gomes Leal et al., 2002). Sections were then rinsed 4 times (3 min each rinse) and DAB reacted according to a previously described protocol (Schnell et al., 1999). After DAB reaction, sections were rinsed 3 times (3 min each) in 0.1 M PBS, dehydrated, and cover-slipped. Some sections were also counterstained with hematoxylin.

In order to investigate whether the rhabdoviruses can induce cell death by apoptosis in experimentally-infected suckling mice, immunohistochemistry for caspase-3, a specific apoptosis marker, was performed (Buki et al., 2000). Primary antibody (Promega, USA) was diluted at 1:250 in PBS. The immunohistochemical procedure was similar to that described above.

2.6. Qualitative analysis

All slides were surveyed using a light microscope (Axiophot, Zeiss, Germany). Images to exemplify the most illustrative fields of different brain regions of virus-infected animals were acquired using a digital camera (Nikon, Coolpix 950, Japan) attached to a microscope (Nikon, Optiphot-2, Japan).

3. Results

Swiss mice submitted to experimental viral infection by nasal instillation presented different average survival times (AST) (Table 1). Rhabdovirus antigens were clearly visible in different brain regions of newborn mice by immunohistochemical assay. Control sections (uninfected animals and slides from infected animal brain sections to which the primary antibody was not added) showed no obvious specific labeling. Similarities and differences regarding CNS tropism of the different viruses could be observed depending on the virus species but all viruses produced fatal encephalitis with neuropathological features of brain inflammation.

3.1. Vesiculoviruses

3.1.1. Carajas virus

Newborn mice infected with Carajas virus presented conspicuous signs of infection by 2 days post-infection (dpi). Animals began to die on the third day pi and the remaining animals were submitted to perfusion. Viral antigens were found in round cells in the meninges, the cortical and cerebellar neurons, and blood vessels. Antigens of this virus were especially present in the cerebellum (Fig. 1A–B). Histopathological analysis revealed that infection with Carajas virus induced both neuronal necrosis and pyknosis (Fig. 2A–B). Necrotic neurons were especially present in some cortical regions, such as visual and parietal cortices. The number of pyknotic cells, as revealed by cresyl violet staining, was apparently higher in cerebellum (Fig. 2B). A number of pyknotic cells were undergoing apoptosis, as confirmed by immunocytochemistry for active caspase-3 (Fig. 2A–B; Fig. 3).

3.1.2. Cocal virus

Animals infected with Cocal virus were more acutely affected than those infected with Carajas virus. The death of these animals occurred about 1 d pi. Viral antigens showed a particular tropism for both perivascular space at the blood vessels (Fig. 1G–H) and cortical white matter (data not shown). Labeled blood vessels were more frequently infected by Cocal virus than by the other viruses tested (Fig. 1H). Cocal virus infection induced both cellular necrosis and pyknosis, mainly in the cerebral cortex and basal ganglia (Fig. 2I–J). Neurons in the final stage of necrosis displayed a completely altered morphology, which was present all over the cerebral cortex (Fig. 2J), and showed a similar to that found for Maraba virus. Some striatal pyknotic cells were also labeled by anti-caspase-3 antibody (Fig. 3C–D).

3.1.3. Maraba virus

More than 25% of Maraba virus-infected animals died by 20 h pi. Histopathological analysis in cresyl violet-stained tissue revealed that the majority of the cortical neurons were found at the final stage of necrosis. These neurons were swollen displaying loss of Nissl substance (Fig. 2C–F). Both necrosis and pyknosis were observed, but there was a clear predominance of the former over the latter (Fig. 2D and F). Necrotic neurons were present in several regions including cerebellum, cerebral cortex, hippocampus, and striatum (Fig. 2C–F). However, there was no cell labeling by anti-active caspase-3 antibody.

Maraba virus antigens were labeled by IHC as clusters of DAB precipitated in several cortical and subcortical areas (Fig. 1K–L). Under higher power magnification, reminiscent debris of previously necrotic cells could be visualized (Fig. 1L). Blood vessel cells and surrounding cells of the choroid plexus were occasionally labeled.

3.1.4. Piry virus

Piry virus induced death around 18 h pi. The pathological outcome was as acute as that induced by the Cocal
Fig. 1. Immunohistochemical labeling of newborn mouse brains experimentally infected with different rhabdoviruses. Cerebellar neurons (arrowhead in B) labeled by anti-Carajas virus antibody (A–B). Round cells (arrowhead in D) labeled by anti-Curionopolis virus antibody in the meninges (C–D). Axonal tracts (arrowhead in F) displaying Itacaiunas virus antigens in the brainstem (E–F). Several blood vessels (arrowhead in H) labeled by Cocal virus immunohistochemistry in the cerebral cortex (G–H). Neuronal cell body and primary dendrites (arrowhead in J) presenting Jurona virus immunoreactivity in the thalamus (I–J). Cellular debris (arrowhead in L) labeled by anti-Maraba antibody in the cerebral cortex (K–L). Cellular debris presenting Piry virus immunoreactivity (arrowhead in N) in the cerebellum (M–N). The scale bars is 200 µm (A, C, E, G, I, K) and 25 µm (B, D, F, H, J, L).
and *Maraba* viruses. Generalized neuronal necrosis was present in both cortical and subcortical structures. The cerebral cortex, striatum, hippocampus, and cerebellum were clearly necrotic. Pyknotic cells were found in these CNS structures but were much more frequent in the cerebral cortex and hippocampus (Fig. 2G–H). IHC for *Piry* virus antigens labeled necrotic profiles preferentially in the cerebellum (Fig. 1M–N). Apoptotic profiles were present in the hippocampus as revealed by immunohistochemistry for active caspase-3 (Fig. 3E–F). Several apoptotic cells were present at the hippocampal stratum oriens in a pattern similar to that found for the pyknotic bodies (Fig. 3F).

3.1.5. *Jurona virus*

*Jurona virus* infection developed a fatal outcome by 36 hpi and induced both necrosis and pyknosis mainly in the cerebral cortex and striatum (Fig. 2K–L). The number of necrotic cells appeared similar to that for *Carajas virus* and clearly less than that for *Maraba virus*. Many pyknotic cells were present in both cerebral cortex and striatum with a clear predominance in the latter (Fig. 2L). The motor cortex appeared to present the highest number of necrotic cells, and interestingly, the cerebellum was apparently unaffected. Pyknotic profiles were not labeled by anti-active caspase-3 immunocytochemistry.

IHC for *Jurona virus* antigens revealed a clear labeling of thalamic structures (Fig. 1I–J). Cell bodies and primary dendrites displaying an apparently normal morphology were clearly identified (Fig. 1J). Neuronal cell bodies were also labeled in other subcortical structures, such as in the midbrain (data not shown). Blood vessels were inconsistently labeled.
Fig. 2. Cerebral necrosis (white arrowheads) and pyknosis (black arrowheads) induced to the brain of intranasally-infected newborn mice by different rhabdoviruses. Necrosis and pyknosis induced by Carajas virus in cerebellum (A–B); Maraba virus in both motor cortex (C–D) and striatum (E–F); Piry virus in the hippocampus (G–H); Cocal virus in the striatum (I–J); Juruna virus in the striatum (K–L); Citoonopolis virus in the cerebral cortex (M–N); and Itacaiunas virus in the brainstem (O–P). Black arrowheads point to pyknotic bodies and the white ones to swollen cells in all high magnification pictures. The sections were stained by the Nissl technique. The scale bar is 200 μm (A, C, E, G, I, K, M, O) and 25 μm (B, D, F, H, J, L, N, P).
3.2. Unassigned rhabdoviruses

3.2.1. Curionopolis virus

Animals infected with Curionopolis virus died around 5–6 d pi. Both pyknotic and necrotic cells were present in a pattern similar to that described for Carajas virus (Fig. 2M–N). IHC labeling was also comparable to that reported for Carajas virus. The cerebellum and meninges were the major structures infected by Curionopolis virus (Fig. 1C–D). In the cerebellum, the...
Fig. 3. Apoptosis observed in newborn mice infected by three different rhabdoviruses. Microphotograph illustrates hematoxylin-stained pyknotic bodies also labeled by immunocytochemistry for active caspase-3 in the brains of newborn mice infected with Carajas (A–B), Cocal (C–D) and Piry (E–F) viruses. Apoptotic cells present in different CNS regions. Arrowheads point to caspase-3 positive pyknotic bodies. The scale bars are 200 μm (A) and 25 μm (B, D, F).

Purkinje cell layer was labeled following infection with this virus (not shown). There was no consistent labeling with anti-active caspase-3.

3.2.2. Itacaiunas virus
Newborn mice infected with Itacaiunas virus presented clinical signs of infection between 8 and 15 dpi. By 8 dpi, some animals presented clinical signs of sickness, but death usually occurred only 15 dpi. Itacaiunas virus antigens could be labeled mostly in axonal tracts in the striatum (Fig. 1E–F), cerebellum and pons. Cell bodies in the cerebellar Purkinje cell layer were also specifically labeled. Histopathological analysis revealed both necrosis and pyknosis (Fig. 2O–P), but apoptosis was not confirmed by anti-caspase-3 immunocytochemistry.

Although the Nissl and hematoxylin stained-pyknotic bodies were also labeled by the anti-caspase-3 antibody, some animals had no pyknotic caspase-3 positive cells. These cells had an apparently normal morphology, similar to those found in subcortical regions of some animals infected with the Curionopolis and Carajas viruses.
4. Discussion

In this study, we have described both immuno-histochemically revealed-neurotropic patterns and neuropathology findings in the immature CNS of mice following intranasal infection with seven Amazonian arboviruses belonging to the *Rhabdoviridae* family (five vesiculoviruses and two unassigned viruses). As expected, all infected mice developed fatal encephalitis. The incubation period, however, was clearly different, depending on the virus species, as demonstrated by different AST (Table 1). Two patterns of rhabdovirus infection were observed. Vesiculoviruses caused fulminant infection, killing animals in a period ranging from 18 to 48 h pi, while unassigned rhabdoviruses caused sub-acute infection and induced death around five days pi or higher for *Curionopolis virus*, and 15 days pi for *Itacaiunas virus*. These differences could be important for both neuropathogenesis and neuroinvasiveness of rhabdoviruses.

The neuropathological findings included pyknosis, necrosis, and apoptosis; the latter confirmed by caspase-3 immunocytochemistry (Figs. 3–4). Both cellular alterations were observed in varying degrees, depending on virus species. Antigens of all viruses were commonly found on blood vessels and meninges, but they were also concentrated in some preferential anatomical regions, depending on the rhabdovirus studied. A more detailed discussion on the most important neuropathological findings is presented below.

4.1. Virus antigen access to CNS

Viruses can get into the CNS through different pathways, which include the axoplasmic transport and a haematogenic pathway during viremia involving viral phagocytosis by mononuclear cells, especially perivascular macrophages (Card et al., 1997, 1998, 1999; Lane et al., 1996). Haematogenic spread of arboviruses to the CNS is more common and may result from an altered blood-brain barrier (BBB) (Whitley and Gnann, 2002). For instance, after an insect bite, a local viral replication occurs in the skin, transient viremia spreads the virus to reticuloendothelial cells and sometimes to skeletal muscles. Secondary viremia improves virus load, and leads to infection of other organs, including the CNS. Once the virus reaches the brain, subsequent replication can remain intraneuronal or can result in intracellular and/or extracellular spread (Whitley and Gnann, 2002).

In the nasal cavity, viruses should infect the respiratory epithelium and propagation to CNS may occur via different neuronal pathways but also through the vasculature (Babic et al., 1994). In the present study, we have not studied the kinetics of viral neuroinvasion, but focused on the conspicuous neuronal labeling in different areas of mouse brain. This is in agreement with previous studies using other viruses, which have described intense neuronal labeling following experimental intranasal infection of different virus species of several families (Babic et al., 1994).

In this study, rhabdoviruses seemed to get into the CNS mainly through the vasculature. Indeed, antigens for virtually all viruses studied were detected on blood vessels in several cerebral regions. In addition, high numbers of immunohistochemically-labeled cells were also present in the meninges of infected litters for at least 5 of the 7 viruses tested (all vesiculoviruses). These cells may comprise monocyte-derived perivascular macrophages. It has been established that monocytes can phagocyte viruses helping these pathogens to get into the CNS...
necrosis and some pyknosis in both motor cortex and pons. On the other hand, in the cerebral cortex and the second in the hippocampus, necrosis and apoptosis, but the first was more frequent.

Maraba and Piry virus species. For instance, Cocal virus also uses an alternative pathway to enter CNS. A similar pattern was also observed for Itacaiunas virus, the olfactory bulb route seems to be the preferential route into the CNS. In fact, it is noteworthy that the granular and mitral cell layers of the olfactory bulb were labeled by Itacaiunas antigens, a virus that induced the longest AST among all viruses studied in the present paper. Nevertheless, Itacaiunas antigens were also present in the axonal tract of motor regions of the brain stem—a zone not directly related to the olfactory system. Thus, it is reasonable to suppose that Itacaiunas virus also uses an alternative pathway to enter CNS. A similar pattern was also observed for Curionopolis virus. Further kinetic studies, using double labeling immunohistochemical protocols should be performed in order to definitely establish the viral entry to the CNS and the possible role of perivascular macrophages in this regard.

4.2. Neuropathological features of Amazonian rhabdoviruses

All experimental mice developed fatal encephalitis with varying AST as to the virus species (Table 1). Necrosis and apoptosis were a common histopathological finding in the infected-mouse brain. The intensities of both cell death and tissue damage were higher for viruses that induced shorter AST, for example, Piry, Maraba and Cocal viruses. These viruses also induced the most acute pathological outcome. For these viruses, infection in suckling mice induced death before 24 h.

The mechanisms of neuronal death are unclear, but they seem to vary depending on the brain region and the arbovirus species. For instance, Piry virus induced both necrosis and apoptosis, but the first was more frequent in the cerebral cortex and the second in the hippocampus. On the other hand, Maraba virus induced intense necrosis and some pyknosis in both motor cortex and striatum, but pyknosis was mainly present in the last region. For the Maraba, Itacaiunas, and Jurona viruses, despite of intense pyknosis revealed by hematoxylin staining, anti-caspase-3 labeling was consistently negative. This confirms that not all pyknotic profiles represent cells undergoing apoptosis as chromatin condensation can also occur in an earlier stage of necrosis (Vila and Przedborski, 2003). Recent studies have confirmed that there is an apoptosis-necrosis continuum with a gradient for these two major forms of cell death (Martin et al., 1998). For Maraba virus, almost all striatal and cortical neurons were found necrotic, which suggests that the time point investigated could be a late one to identify caspase-3 activation. Nevertheless, it cannot be ruled out that apoptosis with caspase-3 activation can be found in earlier survival times. In addition, it has been shown that apoptosis independent of caspase-3 activation can occur in some circumstances (Vila and Przedborski, 2003).

These results suggest that molecular factors inherent to the anatomical region can modulate the type of neuronal death during viral encephalitis. It has been proposed that viruses can induce apoptosis of infected cells either directly, to assist virus dissemination, or by inadvertently triggering cellular sensors that initiate cell death. In these circumstances, apoptotic death would be an innate immune response to viral infection (Everett and McFadden, 1999). Both necrosis and apoptosis can also be a consequence of the inflammatory response induced by the virus infection, as previously suggested (Julkunen et al., 2000; Rempel et al., 2004; Roulston et al., 1999).

We are undertaking experiments in order to address this issue, but both neutrophil recruitment and astrocyte activation occur in the present model of arbovirus infection, as revealed by our preliminary unpublished results. In acute viral encephalitis, capillary and endothelial inflammation of cortical vessels is a striking pathological feature, mostly affecting grey matter or grey/white matter junction. Perivascular lymphocytic infiltration is followed by astrocytosis as disease progresses (Whitley and Gnann, 2002). Neutrotropic viruses that enter the CNS through the vascular route may induce damage to the BBB by activating sentinel macrophages (Khuth et al., 2001). These macrophages could activate matrix metalloproteinases (MMPs) that have been associated with BBB breakdown and tissue destruction (Khuth et al., 2001). It is also known that some viruses infect mouse brain endothelial cells, which increases CNS vascular permeability and results in oedema and more local viral load (Soili Hanninen et al., 1994, 1996, 1997). These mechanisms may be involved in the pathogenesis of neuronal death in the present study.
4.3. Distinct neurotropism of Amazonian arboviruses

Neurotropism, broadly defined as the ability to infect cells within the nervous system, is determined in part by the individual cell type permissive to viral entry and replication, expression of receptor molecules that allow viral entry, and the specific strain of the infecting virus (Patrick et al., 2002).

In this study, arboviruses belonging to the Rhabdoviridae family displayed distinct neurotropism for different newborn mouse brain regions. There was a conspicuous viral neurotropism for motor areas, especially the cerebellum and basal ganglia. That was the case of Carajas, Curionopolis, Cocal, and Piry viruses. Itacaiunas virus displayed a preferential neurotropism for brainstem structures, and the Junona virus, for thalamic structures, while Maraba virus showed a generalized neurotropism. This neurotropism may have clinical significance, as there was a clear correlation between the concentration of viral antigens in these structures and neuropathological outcome. In addition, the Negri bodies, the pathognomonic microscopic finding in human Rabies, are frequently present in hippocampus pyramidal neurons and cerebellum Purkinje cells (Mrak and Young, 1994). These brain regions were affected in the present model of rhabdovirus disease.

Viral neurotropism can be related to neuronal immaturity, as it has been shown for other viruses, such as the Japanese encephalitis virus (Yasui, 2002). In fact, preliminary studies by our group have shown that adult Swiss mice are much less vulnerable to the Amazonian arbovirus pathological effects (unpublished results), which are apparently characterized by mild infections, with the exception of the Maraba and Cocal viruses, which induce death in adult animals in longer AST. The molecular mechanisms by which these viruses or their antigens bind to neurons in specific neuroanatomical regions are unknown, but they may involve molecules like membrane glycoproteins, 65-kDa trypsin-sensitive essential glycoproteins gII and gp50 in transneuronal transfer of pseudorabies virus from the hypoglossal nerves of mice. J. Virol. 75, 11137–11145.

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