MINIREVIEW

How viruses infiltrate the central nervous system

A. MICHALICOVÁ[1], K. BHIDE[3], M. BHIDE[1,3], A. KOVÁČ[1,2*]

[1]Institute of Neuroimmunology Slovak Academy of Sciences, Dúbravská cesta 9, 845 10, Bratislava, Slovak Republic; [2]Department of Pharmacology and Toxicology, The University of Veterinary Medicine and Pharmacy, Komenského 73, 041 81, Košice, Slovak Republic; [3]Laboratory of Biomedical Microbiology and Immunology, The University of Veterinary Medicine and Pharmacy, Košice, Slovak Republic

Summary. – Central nervous system is protected by the blood-brain barrier, which represents a physical, metabolic and transport barrier and is considered to be a part of a highly dynamic system termed neurovascular unit. Several pathogens, among them viruses, are able to invade the brain. Traversal of viruses across the blood-brain barrier is an essential step for the invasion of the central nervous system and can occur by different mechanisms – by paracellular, transcellular and/or by "Trojan horse" pathway. Penetration of viruses to brain can lead to the blood-brain barrier dysfunction, including increased permeability, pleocytosis and encephalopathy. Viruses causing the central nervous system infections include human immunodeficiency virus type 1, rhabdovirus, different flaviviruses, mouse adenovirus type 1, herpes simplex virus, influenza virus, parainfluenza virus, reovirus, lymphocytic choriomeningitis virus, arbovirus, cytomegalovirus, mumps virus, parvovirus B19, measles virus, human T-cell leukemia virus, enterovirus, morbillivirus, bunyaviruses, togaviruses and others. In this review we summarized what is known about the routes of how some viruses enter the brain and how neurons and glial cells react to infection.

Keywords: blood-brain barrier; CNS infection; virus

Contents:

*Corresponding author. E-mail: andrej.kovac@savba.sk; phone: +421-2-54788100-410.
Abbreviations: CNS = central nervous system; BBB = blood-brain barrier; TJs = tight junctions; HIV = human immunodeficiency virus; AIDS = acquired immune deficiency syndrome; RV = rabies virus; WNV = West Nile virus; JEV = Japanese encephalitis virus; TBEV = tick-borne encephalitis virus; MAV-1 = mouse adenovirus type 1

1. Blood-brain barrier and its role in pathogen infections

Infections of central nervous system (CNS) are important cause of morbidity and mortality all over the world (Kim, 2008). Although, CNS is protected by physiological barriers separating the bloodstream and the brain, several pathogens are able to invade the brain (Nassif *et al.*, 2002). The blood-brain barrier (BBB) is a physical, metabolic and transport barrier and it is considered to be a part of a highly dynamic system called neurovascular unit (Kousik *et al.*, 2012; Spindler and Hsu, 2012; Wong *et al.*, 2013). The BBB is formed by brain endothelial cells that line cerebral microvessels

(Abbott *et al.*, 2006). Adjacent endothelial cells are strongly attached to each other by various types of adhesive structures or cell-to-cell junctions, called tight junctions (TJs), which ensure the integrity of the BBB (Michiels, 2003). The proper functioning of the brain depends on functional interactions of endothelial cells with the other cells of the CNS, including neurons, pericytes, mast cells and glia.

Traversal of pathogen across the BBB is an essential step for the invasion of the CNS (Bencurova *et al.*, 2011). Penetration of pathogens to the brain can lead to the BBB dysfunction, including increased permeability, pleocytosis and encephalopathy. Pathogens can cross the BBB by paracellular, transcellular, and/or by "Trojan horse" mechanism (Kim, 2008). Transcellular pathway refers to the penetration of pathogen through the barrier cells without any evidence of microorganisms between the cells or of intracellular damage of TJs. Paracellular pathway is characterized by the microbial penetration between barrier cells with or without TJs damage, while the "Trojan horse" mechanism involves microbial penetration in infected phagocytes. However, most pathogens penetrating the BBB paracellularly or by the "Trojan horse" mechanism are able to cross it also transcellularly (Kim, 2008; Pulzova *et al.*, 2009). Mechanisms of the BBB disruption mediated by paracellular entry of viruses involve alterations in expression or phosphorylation of TJs proteins, disruption of the basal lamina, and disruption of the actin cytoskeleton. The direct implication of viral gene products in BBB disruption is very rare (e.g. human immunodeficiency virus 1).

Among notorious viral agents that cause CNS infections are human immunodeficiency virus type 1, rhabdovirus (rabies), different flaviviruses (West Nile virus, Japanese encephalitis virus, tick-borne encephalitis virus, St. Luis encephalitis virus or Murray Valley encephalitis virus), mouse adenovirus type 1, herpes simplex virus, influenza virus, parainfluenza virus, reovirus, lymphocytic choriomeningitis virus, arbovirus, cytomegalovirus, mumps virus, parvovirus B19, measles virus, human T-cell leukemia virus, enterovirus, morbillivirus (Nipah and Hendra virus), bunyaviruses, togaviruses and others (Pulzova *et al.*, 2009; Spindler and Hsu, 2012).

Transcellular and paracellular permeability of the BBB can be modulated by various factors, such as vasogenic factors, growth factors, cytokines and chemokines, matrix metalloproteinases, free radicals, and lipid mediators. The mechanisms employed by infectious agents to compromise the BBB depend on these factors, and thus the mode of BBB crossing and severity of damage varies a lot. In some cases, the BBB damage can be caused by microbial products only, while in the majority of cases, multiple factors are responsible for the disruption (Spindler and Hsu, 2012). Among this multifactorial interplay, protein-protein interactions between pathogen ligands and host receptors are crucial to trigger the translocation processes, especially through the paracellular and transcellular ways (Bencurova *et al.*, 2011).

2. Human immunodeficiency virus 1

The human immunodeficiency virus (HIV) is a lentivirus belonging to the family of retroviruses (Vigorito *et al.*, 2015). HIV-1 infection affects around 39 million individuals worldwide, of these more than 3 million are children under the age of 15 (Sheets *et al.*, 2016). The clinical signs of acute HIV infection are similar to those in non-HIV infected patients: headache, fever, night sweating, purulent nasal discharge, nasal block, and posterior dripping (Piot and Colebunders, 1987; Sanjar *et al.*, 2011). In later stages, HIV symptoms include weight loss, malaise, fatigue and lethargy, anorexia, abdominal discomfort, diarrhoea, itching, amenorrhea, lymphadenopathy and splenomegaly. Acquired immune deficiency syndrome (AIDS) represents the most severe stage of HIV infection and is characterized by the presence of neurologic abnormalities, opportunistic infections and tumors resulting from a profound cellular immunodeficiency (Piot and Colebunders, 1987).

As far as the molecular processes involved in encephalitis and BBB disruption are concerned, HIV-1 is one of the best-studied viruses (Spindler and Hsu, 2012). HIV replicates by infecting and destroying primarily CD4+ T cells, which are essential for the normal function of the human immune system. The decline of CD4+ T cells causes a progressive immune suppression, resulting in extreme vulnerability to disease and opportunistic infections, like pneumocystis pneumonia and toxoplasmic encephalitis. The terminal stage of HIV viral progression, AIDS, is characterized by the fall of CD4+ cell count below 200/mm^3, and complications with secondary infections (Vigorito *et al.*, 2015). HIV-1 invades the CNS by a "Trojan horse" mechanism, via infected blood cells that cross the BBB either paracellularly or transcellularly (Mishra and Singh, 2014; Spindler and Hsu, 2012). Infected monocytes pass through the BBB during normal turnover of perivascular macrophages or as a result of the production of proinflammatory mediators, like CCL2, which compromise the BBB. Another sources of the BBB infection can be by the penetration of infected CD4+ T cells, direct entrance of the virus or entrance of the virus by transcytosis of brain microvascular endothelium (Ghafouri *et al.*, 2006). After the virus invasion, microglia might become infected and contribute to the production of virus. The HIV-envelope glycoproteins, expressed on the surface of infected cells mediate cell-to-cell fusion with cells that express both CD4+ and HIV co-receptor, resulting in the formation of large multinucleotid giant cells, which also produce virus before they eventually die (Gonzalez-Scarano and Martin-Garcia, 2005) (Fig. 1). The disruption of the BBB is followed by a series of processes caused by the neurotoxic activity of different HIV-1 proteins, such as Tat, gp120, Nef, Vpr and others (Maubert *et al.*, 2015).
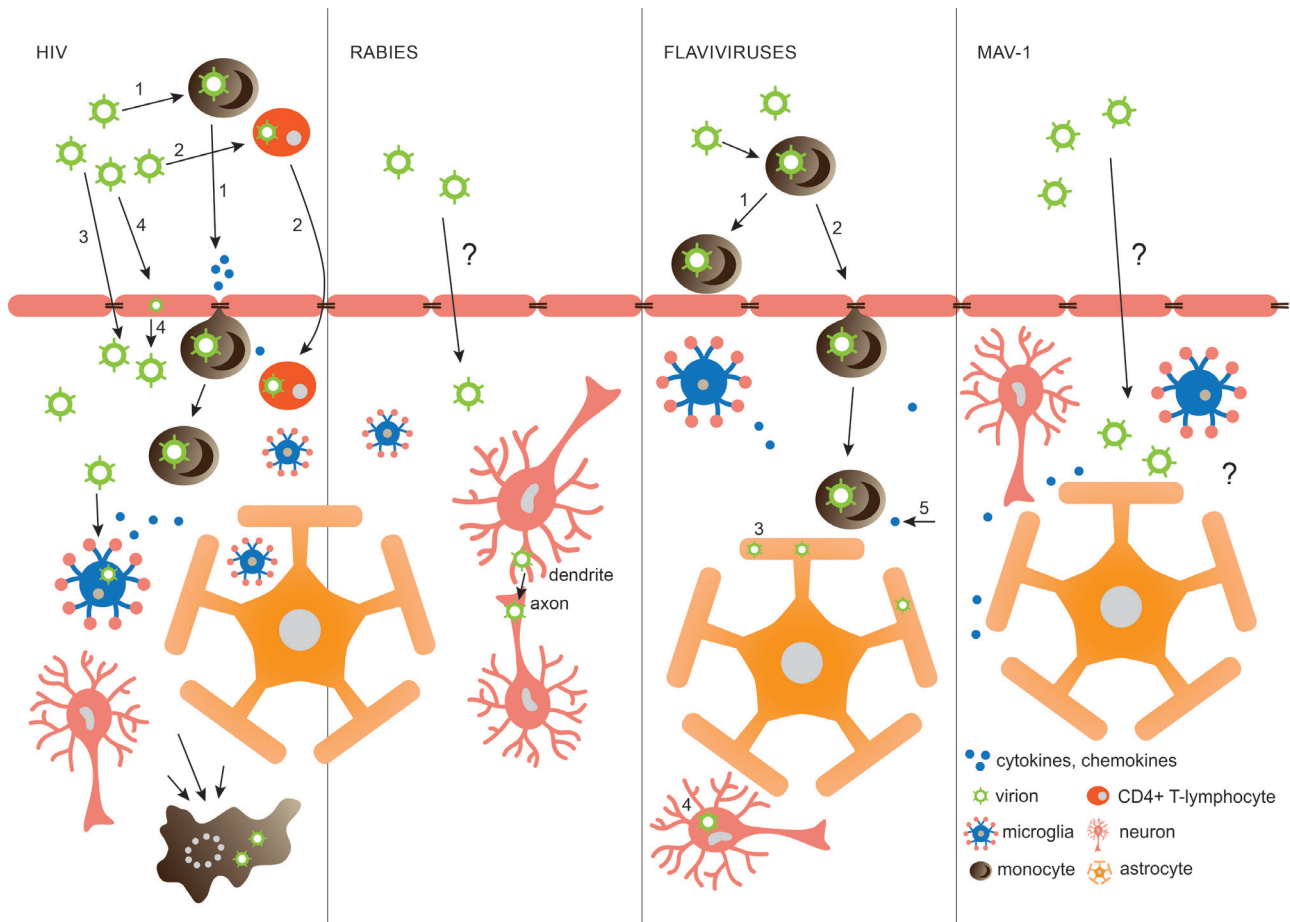
Fig. 1

Routes of viral entry into CNS

HIV-1 utilises a „Trojan horse" mechanism of entry by travelling in infected monocytes (1). These monocytes pass through the BBB during normal turnover of perivascular macrophages or as a result of the production of pro-inflammatory mediators, like CCL2, which compromise the BBB. The penetration of infected CD4+ T cells can be another source of HIV infection in the brain (2). Other probable cause of brain infection might be the direct entrance of the virus (3) or entrance of the virus by transcytosis of brain microvascular endothelial cells (4). After the virus penetration across the BBB, microglia might become infected and contribute to the production of virus. The HIV-envelope glycoproteins, expressed at the surface of infected cells mediate cell-to-cell fusion with cells that express both CD4+ and HIV co-receptor, resulting in the formation of large multinucleated giant cells, which also produce virus before they eventually die. Astrocyte infection is known to be restricted. The mechanism of **rabies** penetration across the BBB is unknown. After the CNS infection, rabies virions are released at the synapse and use retrograde trans-synaptic pathway to infect neighbouring neurons. CNS infection by **flaviviruses** occurs either through the adherence of the virus to brain microvascular endothelial cells (1) or infiltration of infected monocytes across the BBB (2). Viral infiltration causes the infection of BBB and CNS cells. Infection of astrocytes leads to chemokine production facilitating further recruitment of monocytes and macrophages (3). Infected neurons undergo apoptosis and activate the resident microglia which produces an inflammatory response (4). Production of inflammatory cytokines, chemokines, enzymes and matrix-metalloproteinases (5) leads the degradation of endothelial barrier and the release of inflammatory factors recruiting CD4+ and CD8+ T lymphocytes into the CNS parenchyma, what can subsequently lead to further inflammation and damage of the CNS. Encephalitis caused by **MAV-1** is characterized by recruitment of inflammatory cells, secretion of cytokines and chemokines, alteration of tight junctions protein levels and localization in brain endothelial cells. These processes lead to neuroinflammation, neuronal damage and the BBB disruption. The mechanisms used by MAV-1 to penetrate the BBB remain unclear.

3. Rabies virus

The rabies virus (RV) belongs to the genus *Lyssavirus* of the *Rhabdoviridae* family (Paweska *et al.*, 2006). It infects practically all warm-blooded organisms and it spreads to the human through infected saliva by animal bites or scratches. RV remains an important worldwide health problem causing more than 70,000 human deaths annually (Wang *et al.*, 2013). It is endemic in most African and Asian countries (Nel, 2013).

Clinical manifestations of rabies in humans have two forms. The furious form (80 % of infections) is characterized by hydrophobia, excitation with spasm of inspiratory muscles, larynx and pharynx precipitated by attempts to drink and episodes of hallucinations. Animals are often extremely aggressive and they randomly attack objects, other animals or humans. The numb form of rabies (20% of infections) is characterized by weakness and flaccid paralysis, which sometimes causes misdiagnosis at the onset of this clinical form. In both forms, survival after the onset of symptoms is rarely more than 7 days (Schnell *et al*., 2010).

RV is mainly transmitted via saliva following a bite from an infected animal, most often by dogs and cats or through mucous membranes, but not through intact skin (Finnegan *et al*., 2002). Another reservoirs and transmitters of rabies are coyotes, foxes, jackals, mongooses, raccoons, skunks, wolves and bats (Arai, 2005). It has long been suggested that a rabies infection is lethal in humans once the virus reaches the CNS. However, this concept was challenged by an analysis of a small number of rabies patients, revealing that the BBB played a major role in protection against this virus. It has been shown that the main reason for the survival of rabies patients (animals) was enhanced BBB permeability, which allowed immune cells to enter the tissues of the CNS and fight the infection (Roy and Hooper, 2008; Wang *et al*., 2013).

RV pathogenesis is a multigenic trait (Dietzschold *et al*., 2005). RV glycoprotein plays a crucial role in the pathogenesis of RV infection by controlling the rate of virus uptake and trans-synaptic virus spread, and by regulating the rate of virus replication (Dietzschold *et al*., 2008). Moreover, it is suggested that viral elements, regulating gene expression, especially expression of the L gene, are also likely to play a role in RV pathogenesis (Dietzschold *et al*., 2005).

The RV binds to the nicotinic acetylcholine receptors at the neuromuscular junction and travels within motor and sensory axons to the CNS. There is also the spread of the virus from the CNS along neuronal pathways, particularly involving parasympathetic nervous system responsible for the infection of salivary glands, skin, heart and other organs. RV is then secreted into the saliva and can be transmitted to other hosts (Jackson, 2000) (Fig. 1).

4. Flaviviruses (West Nile virus, tick-borne encephalitis virus, Japanese encephalitis virus)

The genus *Flavivirus* of the family *Flaviviridae* consists of more than 70 RNA viruses, involving multiple long known human, animal, and zoonotic pathogens (Ashraf *et al*., 2015; Blazquez *et al*., 2014; Huhtamo *et al*., 2014). They are transmitted by either mosquitoes or ticks and they are the cause of either encephalitis or systemic haemorrhagic septicemia in animals and/or humans (McVey *et al*., 2013).

Nowadays, flaviviruses are found on all continents except Antarctica (Ashhurst *et al*., 2013). As a result of different factors, such as globalization of travel and trade, climate warming, or changes in land use and vector behaviour, different flaviviruses are currently becoming global health problem (Blazquez *et al*., 2014).

Mechanism of passing of flavivirus to the CNS remains incompletely understood, but it is suggested that protein-protein interactions at the BBB may be crucial (Turtle *et al*., 2012). Flaviviruses disrupt the BBB indirectly through the effects of systemic inflammatory cytokines or directly by binding to different structural proteins, like claudins (Neal, 2014). Viral infiltration causes the infection of BBB and CNS cells. Infection of astrocytes leads to chemokine production facilitating further recruitment of monocytes and macrophages. Infected neurons undergo apoptosis and activate the resident microglia, which produce an inflammatory response. Production of inflammatory cytokines (e.g. TNF-α, IL1β, INF-γ and IL-4), chemokines (e.g. CCL2, CCL5, CXCL9, CXCL10), enzymes (COX2) and matrix-metalloproteinases leads the degradation of endothelial barrier and the release of inflammatory factors recruiting CD4+ and CD8+ T lymphocytes into the CNS parenchyma, what can subsequently lead to further inflammation and damage of the CNS (Daep *et al*., 2014) (Fig. 1).

4.1 West Nile virus (WNV)

WNV is a mosquito-borne flavivirus and its transmission cycle occurs between mosquito vectors and reservoir hosts, like aquatic birds. WNV infection in accidental hosts, such as humans or horses, usually results in low level viremia and plays only a little role in this cycle (Suen *et al*., 2014). Human-to-human transmission is possible only through the transfusion of blood or organ transplantation (Di Sabatino *et al*., 2014).

WNV is endemic to numerous parts of Africa, Asia and the Middle East and is now the leading cause of arboviral encephalitis in North America (Ashhurst *et al*., 2013). It is the most widespread member of the Japanese encephalitis virus complex (Di Sabatino *et al*., 2014). WNV infection in humans is in the most cases asymptomatic, but mild influenza-like symptoms may occur. In the most vulnerable categories including elderly, chronically ill, and immunocompromised persons, WNV infection can lead to severe encephalitis and even death. In horses, the course of disease is usually subclinical, but some animals may show neurological symptoms and develop fatal encephalitis (Di Sabatino *et al*., 2014).

The mechanism by which the virus invades the brain is still poorly understood (Suen *et al*., 2014). WNV-associated encephalitis is characterized by the BBB disruption, increased infiltration of cells of immune system into the CNS,

activation of microglia, inflammation and possible loss of neurons. It is also suggested, that WNV may enter into the CNS via transcellular pathway without compromising the BBB. WNV does not induce the cytopathic effect, however induces an expression of claudin-1 and upregulation of VCAM-1 and E-selectin (Pulzova *et al.*, 2009).

Pattern recognition receptor detects the viral RNA (pathogen-associated molecular pattern) and evokes innate immune responses against WNV. Daniels *et al.* demonstrated that WNV pathogen-associated molecular patterns orchestrate endothelial responses to WNV via competing with innate immune cytokine signals at the BBB, which normally prevent the entry of pathogens. While Th1 cytokines increases the BBB permeability, type I interferon induced by WNV promotes and stabilizes its function. Induction of innate cytokines by pattern recognition pathways directly regulates the permeability of the BBB and the formation of TJs via balanced activation of the small GTPases Rac1 and RhoA, which in turn regulated the transendothelial trafficking of the virus. *In vivo* experiments on mice with attenuated type I interferon signalling or interferon induction (Ifnar(-/-) Irf7(-/-)) showed enhanced BBB permeability and TJs dysregulation after WNV infection (Daniels *et al.*, 2014).

4.2 Tick-borne encephalitis virus (TBEV)

TBEV causes severe encephalitis with serious sequel in humans. Unlike other flaviviruses this virus can be transmitted through non-pasteurized milk of infected cows and goats. However, tick remains major vehicle for virus transmission. The *Ixodes* ticks (*I. scapularis*, *I. ricinus* and *I. persulcatus*), most prevalent in Central and Eastern Europe, are the primary vectors of TBEV.

The mechanisms underlying how TBEV gains access to the CNS are not completely elucidated. There are several hypothetical routes for TBEV traversal across BBB. These include cytokine-mediated BBB breakdown, "Trojan horse" theory, and viral entry into the brain microvascular endothelial cells, transcytosis, and the release of virus into the brain parenchyma (Ruzek *et al.*, 2011).

Tick borne encephalitis is commonly recognized by a neurological disorder; however other symptoms like mild fever and itching can also occur. The probability of developing the chronic or permanent neuropsychiatic sequelae is nearly 20% of infected patients (Kaiser, 2008). The virus can infect both meninges and brain. Although, the knowledge on TBEV translocation across the BBB is fragmented, recent research using electron tomography of TBEV infecting neurons has revealed many molecular events that may also take place in brain microvascular endothelial cells infection. The electron tomography revealed direct connections between the tubule-like structures of neurons and viral particles in endoplasmatic reticulum. In the same study, viral particles

were also found in cellular microtubules and vacuoles (Bily *et al.*, 2015).

Apart from neurons TBEV infects astrocytes, which are located between synapses and endothelial cells (Potokar *et al.*, 2014). Astrocytes have several functions in the brain, including neuronal support and the most importantly maintenance of TJs in normal and pathologic conditions (Tao-Cheng *et al.*, 1987). It has been shown previously that TBEV infection alters the permeability of the BBB (Ruzek *et al.*, 2011), and astrocytes may be implicated in this process, since these cells regulate blood flow in the brain (Potokar *et al.*, 2014).

4.3 Japanese encephalitis virus (JEV)

JEV belongs to a complex containing three other viruses – Saint Luis encephalitis virus, Murray Valley encephalitis virus and West Nile virus (Murphy, 1999). It is an etiologic agent causing the Japanese encephalitis. JEV infection is typically inapparent but can cause clinical disease in humans, horses, and swine (McVey *et al.*, 2013).

JEV is endemic throughout Southeastern and Central Asia and results in approximately 30,000–50,000 cases per year (Ashhurst *et al.*, 2013). In humans, the symptoms can range from a mild febrile illness to severe encephalitis, including seizures, a polio-like illness, and different movement disorders. In fatal cases, pathological changes, such as severe degree of vascular congestion, cerebral oedema, neuronal death, astrocyte activation, and microglial proliferation can be observed in various parts of the nervous system.

JEV can be transmitted between animal and human hosts by *Culex* species of mosquitoes. Despite the importance of Japanese encephalitis, only little is known about the pathogenesis of human JEV infection, involving the mechanism of its spread to the CNS and viral tropism within the brain. Since *in vitro* studies have shown that peripheral blood mononuclear cells, including monocytes and macrophages, can be infected and invade the CNS via the antipodal transport of virions or through vascular endothelial cells, it is suggested that JEV may have a peripheral replication cycle. Many flaviviruses have been observed to induce neuronal apoptosis in neurons *in vitro* and in *in vivo* rodent models. Neuronal apoptosis is considered to be one of the hallmarks of neurodegenerative infections. JEV has also been shown to cause loss of neurons due to the rough endoplasmic reticulum stress pathway. Viral tropism in neural progenitor stem cells and immature neurons has also been observed in experimental models of JEV infection. However, mature neurons become resistant to JEV-induced apoptosis because of the increased neuronal expression of cellular inhibitors of apoptosis, like Bcl-2 and Bcl-x.

Additionally to neurons, astrocytes and microglial cells can also be infected. Recent studies of human and mouse

models revealed prominent astrocyte activation, particularly in areas of neuronal damage. Ghoshal *et al.* (2007) reported significant increase of various proinflammatory mediators, such as inducible nitric oxide synthase, cyclooxygenase 2, interleukin 6, interleukin 1b, tumour necrosis factor alpha, and monocyte chemoattractant protein 1, in microglial cells following JEV infection, which may play an important role in inducing neuronal cell death (Zhang *et al.*, 2014).

5. Mouse adenovirus type 1

Mouse adenovirus type 1 (MAV-1) is a non-human-infecting adenovirus belonging to the *Adenoviridae* family of viruses (Hartley and Rowe, 1960; Hsu *et al.*, 2012). MAV-1 infection of immunodeficient mice can result in pneumonia, hepatitis, encephalitis, gastroenteritis, and disseminated disease involving multiple organs (Ashley *et al.*, 2009).

Clinical signs of disease caused by MAV-1 infection in new borne and suckling mice include ruffled coat, lethargy, and terminal burrowing into the cage bedding. Adult mice carrying the severe combined immune deficiency mutation and infected with MAV-1 display also hunching, unsteady gait and poor feeding (Kring *et al.*, 1995).

MAV-1 is similar to human adenovirus in genome and structure, and both viruses cause persistent infections. These properties make MAV-1 a good animal model system for studying adenovirus pathogenesis (Gralinski *et al.*, 2009; Weinberg *et al.*, 2007).

MAV-1 causes both acute and persistent infection in mice and it infects cells of the monocyte/macrophage lineage and brain endothelial cells of the BBB (Guida *et al.*, 1995; Kajon *et al.*, 1998). Infection of endothelial cells is thought to lead to encephalitis. In encephalitis, endothelial cells of the small veins of the meninges and brain are the targets for MAV-1 replication (Charles *et al.*, 1998).

Encephalitis caused by MAV-1 is characterized by recruitment of inflammatory cells, secretion of cytokines and chemokines, and alteration of TJs protein levels and localization in brain endothelial cells (Dallasta *et al.*, 1999; Getts *et al.*, 2008; Gralinski *et al.*, 2009; Chaturvedi *et al.*, 1991; Ivey *et al.*, 2009; Spindler and Hsu, 2012; Verma *et al.*, 2009). These processes lead to neuroinflammation, neuronal damage and the BBB disruption (Gralinski *et al.*, 2009; Charles *et al.*, 1998) (Fig. 1).

6. Conclusions

The above review sums up known information about how different viruses invade the central nervous system. It shows that viruses can cross the blood-brain barrier in a wide variety of infectious diseases. In future research the mechanisms of penetration of the viruses across the blood-brain barrier should be explored as a therapeutic strategy against viral infections of CNS.

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