Chapter 6

Arboviral Encephalitis

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Additional information is available at the end of the chapter

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1. Introduction

Arboviruses (arthropod-borne viruses) are a group of pathogens that are transmitted by hematophagous arthropods, mainly mosquitoes and ticks, between susceptible vertebrates [1]; many of them are also characterized by their movement through arthropod communities: vertical (or transovarial) [2] and venereal transmission [3]. Thus far, more than 500 arboviruses have been identified worldwide, particularly in tropical and subtropical areas [4-6]. Of these, some 80 species can cause human diseases with a broad spectrum of symptoms, including encephalitis, fever, and hemorrhaging [7]. Most arboviruses are classified into three families (the Togaviridae, Flaviviridae, and Bunyaviridae) in the current viral classification system. Minor groups of arboviruses include those belonging to the Rhabdoviridae (vesicular stomatitis Indian and bovine ephemeral fever viruses), Reoviridae (bluetongue virus and Colorado tick fever), and Asfarviridae (African swine fever virus; ASFV); all of which have trivial or no roles in causing human diseases.

Viruses belonging to the Togaviridae are enveloped and spherical with a size of 65~70 nm in diameter; they contain an icosahedral nucleocapsid within which is included single-stranded positive-sense RNA [8]. Viral RNA serves as both the genome and viral messenger (m)RNA. The entire genome encodes a non-structural polyprotein which is processed by host and viral proteases, while a structural polyprotein is expressed by subgenomic mRNA [9]. The genus *Alphavirus* in the family Togaviridae includes 29 virus species, all of which are transmitted by mosquitoes [10]. The Flaviviridae is composed of viruses that also contain single-stranded positive-sense RNA; however, their virions are smaller in size than Alphaviruses, usually 45~50 nm in diameter [11]. The genus *Flavivirus* contains about 70 members; a number of them are infectious to humans, *e.g.*, dengue virus and West Nile (WN) virus. Flaviviral RNA possesses a single open reading frame, encoding a polyprotein, which is then processed to three structural proteins (C, M, and E) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) by host and viral proteases [11].

The Bunyaviridae is one of the largest groupings of animal viruses, containing more than 300 viruses [12]. Except for the genus *Hantavirus*, all of them are transmitted by arthropods [12]. Viral particles are spherical with a size >100 nm in diameter, and are composed of four structural proteins encoded on its tripartite single-stranded negative-sense RNA genome consisting of the L, M, and S segments [13].

Various arboviruses belonging to those three major families can specifically cause encephalitis. Of these, Eastern equine encephalitis (EEE) virus, Western equine encephalitis (WEE) virus, and Venezuelan encephalitis (VEE) virus belong to the Togaviridae [14], Japanese encephalitis (JE) virus, St. Louis encephalitis (SLE) virus, WN virus, and tick-borne encephalitis (TBE) virus are from the Flaviviridae [15, 16], while California encephalitis (CE) virus and La Crosse (LAC) virus are members of the Bunyaviridae [7]. Recently, increasing evidence has shown that certain arboviruses such as dengue (DENV) and chikungunya viruses (CHIKV) may occasionally cause encephalitis in addition to their conventional symptoms,which usually involves headaches, muscle and joint pain, and rashes [17-19].

2. Epidemiology of encephalitic arboviruses

Arboviruses are usually transmitted through bites of blood-feeding arthropods (primarily mosquitoes and ticks) in two major cycles (Figure 1). The man-arthropod-man cycle is characteristic of dengue virus, while EEE, WEE, WN, JE, and CE viruses are transmitted by an alternative cycle involving non-human mammals and birds [10]. For the infections by arboviruses that cause encephalitis, humans or horses become an incidental or dead-end host, while animals such as birds and pigs serve as reservoirs or amplifying hosts [20].

Togaviridae. Viruses causing EEE, WEE, and VEE are all members of the Alphavirus genus in the family Togaviridae [21]. In fact, they are the only viruses in this group that commonly cause encephalitis and are restricted to the Americas. There are other Alphaviruses also with limited distributions, such as CHIKV (Asia and Africa), O'nyong-nyong virus (Africa), Sindbis virus (Africa, Europe, and Asia), Mayaro virus (South America), and Ross River virus (Australia), however these are expected to eventually become distributed worldwide [22]. Epidemiologically, all Togaviridae are similar in that these viruses have wild avian hosts, are transmitted from birds to mammals by mosquitoes, and may cause encephalitis in horses and humans [22].

Flaviviridae. At least 7 arboviruses including TBE, Kyasanur Forest disease (KFD), JE, Murray Valley encephalitis (MVE), SLE, Rocio, and WN viruses are reported to be associated with causing encephalitic symptoms [23]. Some of these are described below.

The TBE virus is a member of the family Flaviviridae, which is geographically distributed worldwide, usually in rural areas at temperate latitudes, including all over Europe and the Scandinavia, the former Soviet Union, and East Asia [24]. Incidences of human cases markedly increased in the early 1990s, mostly in Europe [25]. It was reported that the TBE incidence was 8690 cases during 1965~1992, while 8674 cases were documented in a smaller

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window of time between 1993 and 2006 in the Czech Republic, indicating a steep rise in this region [25]. Rodents are the primary reservoir hosts of this virus, which is transmitted by the bites of hard ticks (*Ixodes*) in nature [24].

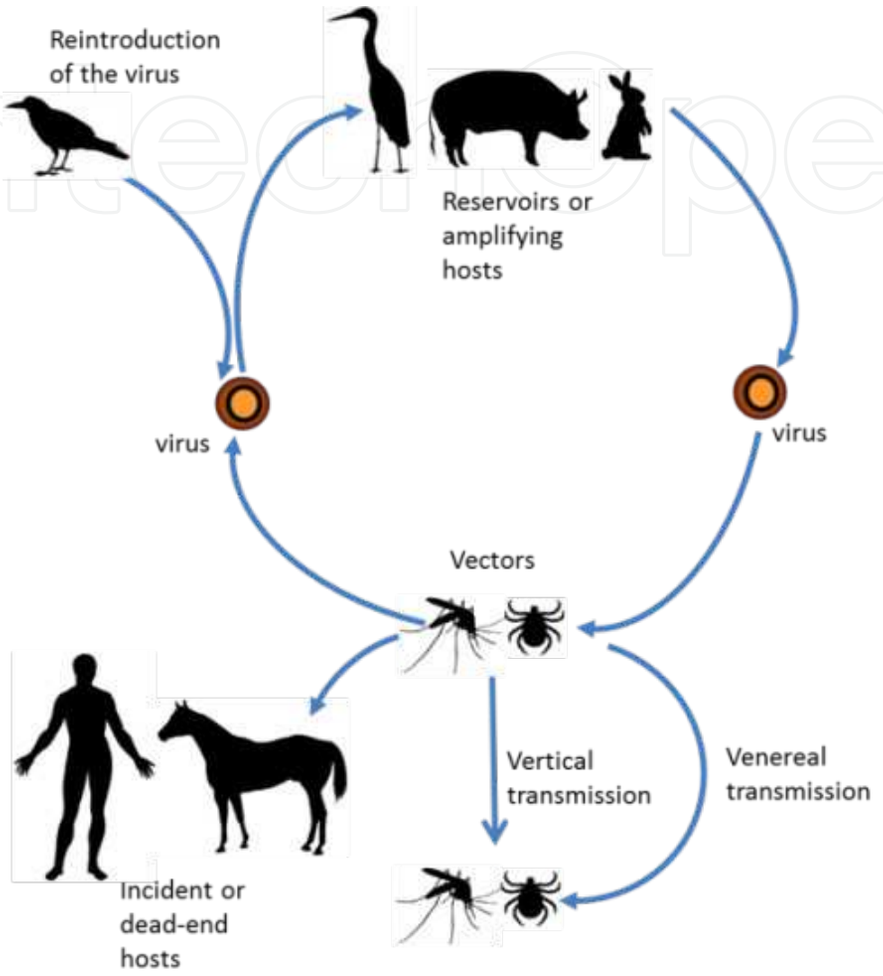

Figure 1. Transmission cycles of arboviruses in nature. Two major cycles cover the transmission of most arboviruses, one is mam-to-man and the other usually involves non-human mammals and birds.

The JE virus is mainly amplified in pigs and birds and are transmitted by *Culex* mosquitoes (primarily *Cx. tritaeneorhunchus*) between vertebrates [26]; it causes a significant number of human encephalitis cases in most areas of Asia, especially eastern, southern, and southeastern Asia, as well as the South Pacific regions [27]. It recently expanded to the Torres Strait of northern Australia in 1999, and has now become endemic in Australia [28, 29]. JE virus is estimated to cause about 30,000~50,000 cases each year worldwide [15, 30]; of which, 10,000~15,000 may be fatal [31].

WN virus was first isolated from a febrile patient in the West Nile region of Uganda in 1937 [32]. It has caused epidemics in Africa, Europe, the Middle East, Asia, and, more recently, in North America [33]. Since the emergence of WN virus in the United States in 1999, it has

spread all over North America and caused more than 20,000 humans to be ill and 770 deaths (http://www.cdc.gov/ncidod/dvbid/westnile/surv and control.htm). Neuroinvasive disease due to WN virus infection can occur, 2946 and 2866 cases were reported in 2002 and 2003, respectively [34].

The SLE virus is a close relative to WN virus, and actually is a member of the Japanese encephalitis serocomplex [35]. Predominantly, SLE virus is naturally maintained in a transmission cycle between ornithophilic mosquitoes and birds, but occasionally these arthropods feed on mammalian blood, causing encephalitis in humans [36]. Nearly 5000 human infections were reported between 1964 and 2005, making it the major cause of epidemic encephalitis in association with flaviviral infections before the introduction of WN virus into the United States (http://www.cdc.gov/ncidod/dvbid/arbor/pdf/SLEDOC07132006.pdf).

Buynaviridae. In this family, viruses involving symptoms of encephalitis include Rift Valley fever (RVF), LAC, CE, and Jamestown Canyon [37]; all are mosquito-borne. RVF virus mostly occurs in Africa and the Middle East, while the other three, which are classified in the California serogroup, are restrictedly distributed in North America [37]. Of these, the LAC virus causes the most human disease, with dozens to hundreds of hospitalized cases reported each year in the United States [38]; unlike EEE, California serogroup including LAC is not dependent on avian hosts for natural transmission. Rodents usually serve as its major vertebrate host [37].

3. Mechanism of central nervous system (CNS) infection by arboviruses

Despite many years of intensive efforts and investigations on the pathways leading to infections of the CNS by arboviral families after the bite of an arthropod carrying an infectious agent, the exact mechanism remains to be further delineated. There are multiple routes that can be considered, depending on the characteristics of the virus. Some advocate the mechanism of direct viral spread from the periphery to the CNS [39], particularly for arboviruses involved in brain infections. It is thought that these viruses are amplified in dermal tissues and then in lymph nodes via migration of dendritic (Langerhans) cells before invading the CNS [40, 41]. However, the mechanism allowing for these viruses to perform the last step, to enter and invade the CNS, is less clear. The MVE, SLE, and JE viruses were speculated to enter the CNS via the olfactory pathway [42], while transcytosis across cerebral capillary endothelial cells was reported in JE [43]. In addition, virion-budding on the parenchymal cells after replication at the blood-brain barrier may also occur [44]. In experimental models, many infections by encephalitic arboviruses are diffusely spread throughout the brain [45, 46]. Furthermore, the absence of viral antigens in the choroid plexus or ependyma indicate that these viruses were not actively targeted to and replicated in this tissue but rather entered the CNS via a hematogenous route [47] (Figure 2), especially in patients with severe viremia [48].

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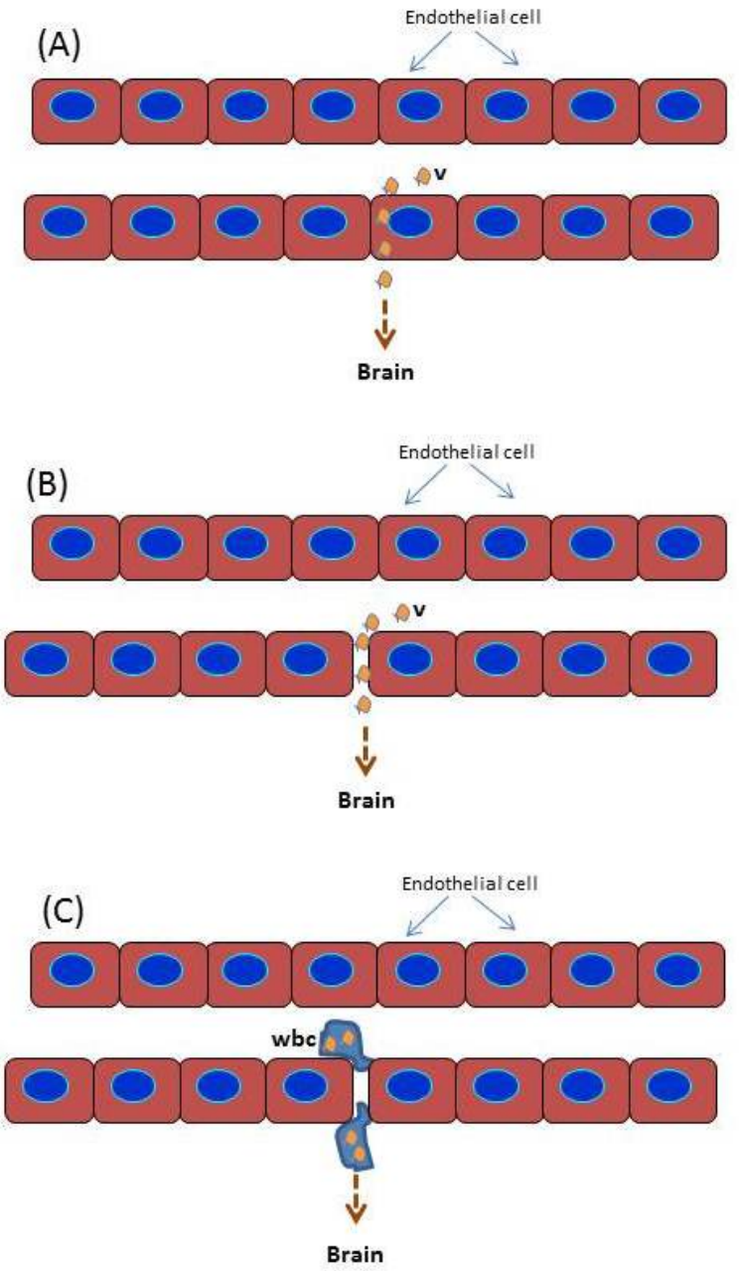

Figure 2. Hypothetic routes for arboviruses to infect the brain tissue hematogenously. (A) Infection of endothelial cells before the virion enters the brain tissue. (B) Virions enter the brain tissue through disrupted BBB. (C) Infected white blood cells enter the brain tissue by passing through the disrupted BBB.

In a study on JE, extensive infection of neurons resulting in cellular defects was shown in the cerebrum and cerebellum [49]. The cerebral and cerebellar capillary endothelial cells are responsible for maintaining the integrity of the blood-brain barrier (BBB) [50]. In both animals and humans, the BBB generally prevents viral invasion into the CNS [51], unless it has been disrupted, resulting in increased permeability and inflammatory cell infiltration [52, 53]. Disruptions in the BBB actually allows for peripheral blood mononuclear cells (PBMCs) to migrate from the circulation into brain tissues [54, 55].

Under normal circumstances, lymphocytes constantly enter the CNS, but in small numbers [56]. However their presence in the CNS may increase in response to viral infections [57]. In fact, infected PBMCs can be isolated in brains from mice inoculated with JE virus as early as 3 days post-infection [58]. Moreover, leukocytes were observed moving between endothelial cells of capillaries at sites in the BBB where tight junctions had been dissociated [49]. This suggests that at least some inflammatory leukocytes that had become infected in the periphery move along in the blood current and migrate to the CNS tissues [58, 59]. Furthermore, infection and resultant apoptosis of astrocytes, which serve as a protective component of the BBB and can defend against penetrated virions or virus-infected leukocytes, are frequently seen in the brain. This probably results in severe impairment of the BBB, facilitating the passage of more virus-infected PBMCs, using a "Trojan horse" strategy.

4. Pathogenesis of arboviral infections

Arboviral diseases start with a bite from an arthropod creature carrying infectious virus. The pathogen may be considered an innocent bystander or an unnecessary byproduct from an infected vertebrate host. The arthropod imbibes this blood for its own purposes, to facilitate ovulation, and takes up the accompanying virus in the meal. The presence of pathogen is not a critical event in the life cycle of the insect and may or may not cause it harm. The persistence of disease is none of these creatures' fault, since survival is the game plan for all organisms on earth. In many instances, arboviruses are capable of surviving inside the coming host without inducing any visible adverse effects. Given the opportunity, the pathogens will reentry and challenge a new host. If the host is capable of implementing a "survival strategy" in response to the viral infection, the host will be fine. Occasionally, these creatures may enter a host, such as human beings, in which the environment may not be as friendly as others, and a hostile survival race is engaged. The race tactics instigated by both sides are normally controllable and do not result in overt disease. But in some cases, the regulatory programs in the host do not coordinate well with each other or could also be disturbed and/or handcuffed by substances released from the pathogens. This can result in dysfunctional operational systems that are harmful to the host, leading to detrimental outcomes, including death. As a whole, the occurrence of the severe consequences is very rare. For instance, with JEV infection, the overall global incidence of cases annually is at 1.8 per 100,000 people [60].

Timing is critical in the diagnosis of acute arboviral encephalitis. The progression and variation in clinical manifestations among infected subjects may differ, depending on the individual's age and geographical habitat, the arthropod's feeding behavior, genetic differences in the viral strain, and the immune status of the affected patients. One of the common clinical features in arboviral infections is viremia. However, the duration and level of this viremia in humans is significantly different with each and every family of viruses. In a commensal arboviral-host relationship, one may expect high levels of viremia to cause too much pathogenesis in the host but too low levels to not facilitate transmission. One may expect a consistent middle range in viremia to be obtained. Extreme variation in or high titers of vi-

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rus in the blood may be a sign that humans are an accidental or dead-end host to most arboviruses. Identifying the cellular sources responsible for viremia will likely help us uncover the underlying mechanisms leading to arboviral encephalitis and aid in the development of vaccines and anti-viral drugs. Because of this, finding the permissive cell lineages accounting for circulating virus in infected patients has been the central focus for several decades. In spite of these efforts, the answer remains elusive.

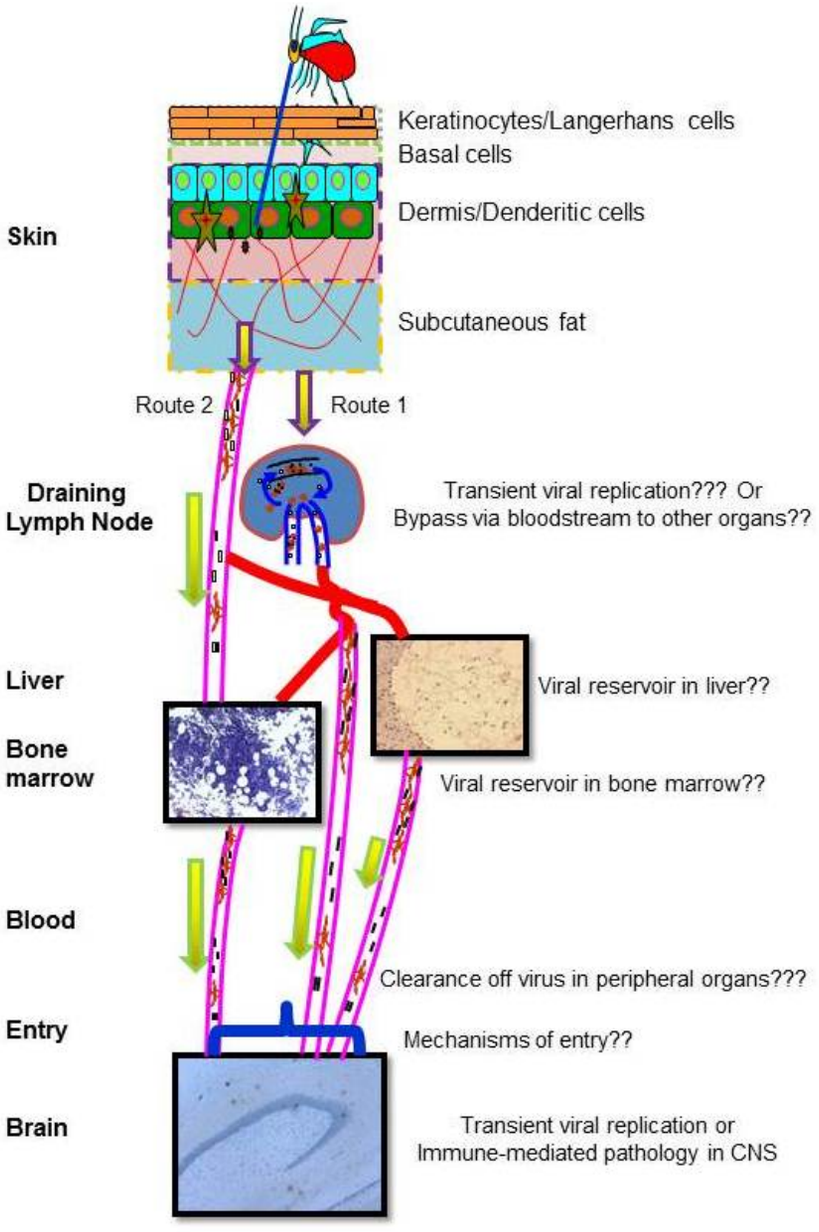

Figure 3. The possible route of the virus in vertebrates from peripheral tissues to the brain. Arboviral infections start with the bite of insects carrying an infectious virus. The exact location where the virus is deposited remains poorly understood. There are multiple ways a virus may spread and circulate before reaching to the brain. Please refer to the text for more details.

Arboviral infections are introduced into the hosts during the blood meals of arthropods carrying infectious virus. The first obstacle that the arthropod encounters is the physical barrier of the skin, which is composed of several layers of keratinocytes interspersed with a network of capillaries (Figure 3). There are two possible routes that the virus may use as a reservoir to amplify the progeny after its deposition by the mosquito. One passage way may be released into the blood pools of lacerated capillaries. In this situation, it is generally assumed that the initial target cell supporting the viral replication is Langerhans dendritic cells of the skin (Figure 3, route 1) [61]. The infected Langerhans dendritic cells migrate to draining lymph nodes where a brief viral replication may occur and the virus is considered to enter the blood stream through the lymphatic and thoracic ducts [61]. The virus may enter the bone marrow [62] or liver [63] where a secondary amplification may occur or directly disseminate to the brain inducing inflammation.

An alternate route would be direct deposition of the viruses into the blood stream (Figure 3, route 2), or so-called capillary feeding, during the engorgement of the arthropod. Results from RVFV suggest that the liver seems to be an early and dominant target of the virus [63]. The damage to the hepatocytes of the RVFV-infected liver is likely a result of apoptosis [63]. The evidence suggests that this virus may get deposit directly into the capillary and take a ride through the circulation to the liver compartment where permissive cells, likely hepatocytes, provide RVFV a means to produce progeny (Figure 3). In addition, studies investigating mosquito imbibing behavior with *Aedes aegypti* revealed that the mosquito's proboscis is flexible and predominantly obtains blood directly from the capillary and only occasionally from the blood pools formed in the tissues by the leakage from previously lacerated capillaries [64]. These results were later confirmed with the mouse's ear and human beings implementing the same experimental designs [65, 66]. In this route (Figure 3, route 2), the virus may gain access directly to the bone marrow where a brief viral replication can occur, extravasates into the circulation, disseminates to other parts of the body, and penetrates the brain via mechanisms discussed in Figure 2.

However, determining the first cells infected by the viruses subsequent to the bite remains a challenging event to investigators. The scenario via route 1 (Figure 3) is complicated by a number of issues. Keratinocytes on the outermost epidermal layer of the skin are endowed with toll-like-receptors (TLR) [67] and may be considered a component of the primary innate immune system. Langerhans cells mainly reside in the thin layer of the epidermis, which does not contain capillaries, while the dendritic cells are predominantly in the thicker dermis layer, which is filled with capillaries. Route 1 has been extensively investigated with diseases derived from mosquito-borne viruses. This pathway could be the true route for those viruses belonging to the human-is-dead-end-host group, since the virus titers from these cells are too low to permit transmission to new mosquitoes. In contrast, if human beings are the host for the virus, such as dengue virus, then the assumption that this virus takes this route should be reconsidered. Experiments have revealed that only a very short window period is available for dengue virus to be transmitted, during the high viremic stage, usually within 3-5 days after the onset of the clinical fever. Thus, if the mosquitoes imbibe the blood meal during this stage, the virus will spillover and infect the local Langer-

hans dendritic cells and the cycle of illness will resume. If this is the case, then we would observe a sinusoidal wave-like pattern for viremia in infected dengue patients. But in reality, this is not the case. Thus, this evidence indicates that an alternate route could exist, such as direct deposition of virus into the blood stream. Interestingly, it has been suggested that during imbibing, approximately 50% of the fascicle penetrates into the skin [68], suggesting that the location of the blood drawn by the vector is from the capillary-rich dermis layer, implicating that pathogens may be directly injected into the blood.

One of the puzzling issues is what cellular constituents are the protective components in asymptomatic cases. Interestingly, apoptotic keratinocytes and dendritic cells are observed in human skin explants when dengue virus is directly injected into the epidermis with a fine needle [69]. Considering the fact that a majority of dengue virus infections are asymptomatic, this evidence suggests that the role of dendritic cells at the site of fascicle penetration is to eliminate or temporarily contain the intruders and thereby prevent or reduce the dissemination of dengue virus. However, the role of keratinocytes and dendritic cells in clearance of dengue virus remains to be further investigated.

Although most persons bitten by an infected mosquito will experience no symptoms or will have a very mild presentation of the disease, approximately 1 to 2 percent will develop a recognizable illness. The clinical symptoms for the initial phase of arboviral encephalitis are very similar and similarly variable from person-to-person for all the virus families. Some individuals may have mild symptoms, such as a fever and headache, while others may have a more severe presentation. In this case, symptoms may include a rapid onset of severe headache, high fever, muscle aches, stiffness in the back of the neck, and problems with muscle coordination, disorientation, photophobia, convulsions and coma. The illness will usually occur five to 15 days after the bite of an infected mosquito or tick. However, the symptoms may resemble other common febrile illnesses. Thus, in order to diagnose correctly and determine the proper treatment in a timely manner, it is important to seek professional help immediately or as soon as clinical signs appear.

In order for an affected subject to have a risk for neurological disorder, the virus entering the human host has to possess two major criteria: neuroinvasiveness and neruovirulence. The term “neuroinvasiveness” means that the virus is capable of passing or crossing through the BBB, a structure that separates the immune privileged compartment of the brain from the peripheral system. The term “neruovirulence” refers to the capacity of viral replication in the CNS tissues. There are several mechanisms involved on the induction of neuroinvasion. The virus can either replicate and induced damage of the nearby endothelial cells [70] in the cerebral capillary or in striated muscle [71] surrounding the BBB. Alternatively, virus may enter the CNS by endocytosis via the olfactory bulb or the choroid plexus, for example, JEV [43], CHIKV [72] and VEEV [73]. In addition, high viremia is a major feature of only some of the arboviral infections, thus some viruses can cross the BBB via the vascular route by passive transfer carried by infected leukocytes [74]. Spreading virus to the CNS through the trigeminal nerve after local amplification of the virus has been proposed as well [73]. The neurological symptoms induced by some of these arboviruses, which are able to increase the permeability of vasculature and spillover into the CNS, are capable of disrupt-

ing cognitive biological processes. In order to differentiate the evasion strategies employed, animal models are required. Currently, there are only a limited number of animal models available for a few arboviruses; JEV [59], EEEV [75], LACV [71], WNV [76] and CHIKV [72]. However, the cardinal features of human clinical encephalitis induced by these arboviruses are hardly reproduced in these models. Therefore, what the exact mechanisms by which arboviruses cross the BBB remains poorly understood, as well as the precise mechanisms by which circulating peripheral pathogens induce the inflammation of the brain remain largely unknown.

Nevertheless, the best systems available that have been used to characterize the biological properties of arboviruses in animal models are the WNV [76, 77], LACV [71], EEEV [75], and CHIKV [72, 78]. Results revealed that viral strain variations, in addition to the host age and immune conditions, contribute a significantly to neuroinvasiveness and neurovirulence. Infection of the mice intradermally or subcutaneously leads to the robust replication of WNV, LACV, and CHIKV in the brain, particularly in newborn mice. But the mechanisms contributing to neurotropism of other viruses are less clear since suitable models are not available.

When viruses enter the CNS, a variety of cells are permissive for infection [46, 79]; some cells may be more susceptible than others, and the viruses may have their differential preferences [74, 75, 78, 80]. Regardless, the net consequence is the activation and/or damage to residential cells. This results in the recruitment of defense cells with immune system functions to the damaged site. An inflammatory response occurs due to the presence of an overproduction of multiple functional cytokines from the infiltrating cells [81-83]. The nature of the privileged environment of the brain bestows it with characteristics that make restoration to the default normal status far more complicated than other parts of the body. The most salient feature of the brain is that a large proportion of the cells are terminally differentiated. These cells are very difficult to renew and replace. Therefore, affected encephalitic patients suffer long-term neurological impairment as a result from the infection [18, 28]. These symptoms include short-term or long-term memory loss, seizures, and impaired judgment [28, 84, 85]. A neurological exam is performed to evaluate the mental status, detect neurological problems, such as motor dysfunction and seizures, and help determine which area of the brain is affected [18].

The causes of the dysfunctional circuitry in neurons are likely different among the arboviruses. Some viruses have the capacity of direct engagement with neurons by infection, while others may induce cell death or apoptosis in nearby cells, which shed releasates, likely triggering a cascade of events that damages the neuronal tissue [81, 82]. This may be why some viruses can be recovered from the CNS easier than others in autopsy specimens. For those viruses capable of infecting small animals, results also suggest the observed scenarios. In contrast, for the viruses with limited capacity to replicate in animal models, the actual causes of neurological symptoms are less clear.

The initial symptoms of the arbovirus infections that induced encephalitis are very similar, especially for those mild cases of encephalitis, which makes the correct diagnosis a challenge to physicians. In order for accurate diagnosis, in addition to the routine examination on the physical performance, specific tests are required, such as electroencephalogram, brain mag-

netic resonance imaging (MRI) and X-ray computed tomography (CT). These tests allow for a scan of the head to detect abnormalities, such as swelling (edema) and bleeding (hemorrhage) [86]. These sophisticated instruments are likely available in very advanced clinics and may not be very convenient or available for the majority of patients affected by arboviral encephalitis. Thus, alternate diagnostic methods are applied. These are biological approaches, which include virus isolation from cerebrospinal fluid, blood, and biopsy specimens, detection of viral genetic and/or antigenic materials, and specific antibodies to the virus. However, there are pros and cons for each of these diagnostic assays. Sensitivity and specificity, and antibody cross-reactivity are always a concern.

5. Treatment of arboviral infections

Currently used drugs to treat arboviral encephalitis. There is no cure for arboviral encephalitis and treatment is generally supportive, with maintenance of respiratory and circulatory systems while the infection runs its course. The purpose of the palliative care is to reduce the malfunctioning of critical organs and to relieve symptoms, while the body fights the infection. The priority of the treatment is to ensure the alleviation of pain, as well as to mitigate the swelling in the brain, reduce the fever and prevent dehydration and other chemical imbalances by administration of intravenous fluids. As a whole, the treatment for arboviral encephalitis depends on the cause. Some clinical cases of arboviral encephalitis can be mitigated successfully if medication is started as soon as possible. A number of therapeutic drugs specific to arboviral infections are under investigation for their potential antiviral and neuroprotective effects: minocycline and curcumin for JEV and other arboviruses [87-89], ribavirin for LACV [90], interferon (Omr-IgG-aM) and humanized monoclonal antibody (Mab E16) as a potential candidate for WNV treatment [61, 91, 92]. However, currently there is limited information available on the effectiveness of these therapeutic modalities in the clinical setting. Additionally, there are a number of reliable medicines that are commonly prescribed to treat the symptoms mentioned above; administration of benzodiazepines (*e.g.*, lorazepam [Ativan®) to prevent seizure, diuretics drugs (*e.g.*, furosemide or mannitol) to reduce brain swelling, sedatives to relieve irritability, antibiotics to prevent secondary infections, and acetaminophen to control fever and headache. For those patients whose brain functions may be severely affected, interventions like physical therapy and speech therapy may be needed after the illness is controlled.

6. New drug development

The life cycle of arboviruses *in vivo* is not well understood, even though a great amount of detail on the comprehensive biology of these viruses *in vitro* has been intensively investigated and uncovered. As aforementioned, the genetic material for a majority of the arbovriuses is positive-sense single-stranded RNA, which can function as mRNA and be infectious by itself. It has been proposed that this genomic viral RNA can become encapsulated within the

biological material from the host cell to form an infectious vesicle.These particles may fuse with other biologically functional identities, potentially leading to the initiation of new infections, which can result in the formation of completed and perfect virions. Interference with the processes and network signaling involving classical virion formation has been a common target for drug development. However, in reality, the perfect virion *in vivo* has not been visualized, suggesting an alternate form of virion may exist *in vivo*. Consequently, the real structures needed to design the intervention remains elusive. Furthermore, diseases induced by arboviruses are acute illnesses where timing is critical. Infected individuals normally delay in seeking professional help, resulting in the subjects arriving at the hospital in a far worsen condition. Thus, the availability of intervention drugs, the timing of the administration and the effect of the drugs on the arboviral infections remain critical issues.

7. Prognosis

Prognosis depends on the particular type of arbovirus causing disease, and on the age and prior health status of the patient. The prognosis is worse in very young patients, elderly patients, and patients with compromised immune systems. LAC encephalitis most often occurs in children, while WNV and SLV encephalitis usually occur in persons older than 50 years of age [20]. Encephalitis caused by EEV and JEV carries a high risk for serious neurological damage and death. Death rates range all the way up to 20% for arboviral encephalitis, and the rates of lifelong effects due to brain damage can reach 60% for some types of arboviruses.

8. Vaccine prevention for arboviral infections

Infection with an arbovirus provides immunity to that specific virus, but not to other arboviruses, suggesting that arboviral infection is a vaccine preventable disease. Thus, the development of new, more effective vaccines and the appropriate animal models in which to test them are paramount. Although for many important arboviruses, there are currently no approved vaccines available for human use, while for some, safe and effective vaccines have been used for decades. For instance, a clinical approved inactivated vaccine against TBEV has been used in Russia, Germany, Austria, and China [93].

JEV is one of the few arboviruses for which a vaccine is available. The JEV vaccine made from infected mouse brain can achieve efficacies of at least 80% [94]. But because of the cost and safety concerns, development of a better JEV vaccine has been an ongoing project. For example, the development of a live-attenuated virus vaccine (SA14-14-2, for use in China and a part of Asia) and more recently, in March 2009, the FDA approved a new, inactivated cell-culture-derived JEV vaccine (IXIARO) for use in adult travelers over the age of 17 [95-98]. In addition, a live-attenuated yellow fever–Japanese encephalitis chimeric vaccine (IMOJEV™) was recently licensed in Australia and is under review in Thailand [98].

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As for WNV, an approved and efficacious vaccine for humans is not available, even though equine WNV vaccines are in use [99]. However, it is anticipated that a WNV vaccine for human use will be available within a couple of years. In addition, inactivated TBEV vaccine is currently available in Europe [100].

For others, such as the Alphaviruses, human vaccines are available only as Investigational New Drugs, and thus are not in widespread use.The rest of the arboviral vaccines are currently undergoing clinical phase III trials, and are anticipated to be available for public usage within 5 years if everything goes as planned. While some of these vaccines have currently only received approval for animal usage, newer versions for human use are in the process of being evaluated or developed.

New challenges in vaccine development have been met with new technologies in vaccine research. Many of the newer vaccines are now being developed by recombinant DNA technology [100]. For example, chimeric virus vaccines have been developed using infectious clone technology for many arboviruses including, WNV, JEV, and TBEV. Other successful approaches have involved the use of naked DNA encoding and subsequently expressing the desired protective epitopes. Naked DNA vaccines have been used for TBEV and JEV and are currently under development for use against WNV. The development of less expensive, more authentic animal models to evaluate new vaccines against arboviral diseases will become increasingly important as these new approaches in vaccine research are realized.

However, technical issues do exist in the nature of these viruses. One of the unique biological features in a majority of arboviruses is the constitution of the genetic material. The positive-sense single-stranded RNA genome can function as mRNA, which is capable of producing an infectious virus if the RNA is inside a biologically functional identity. To add the second layer of difficulty in vaccine development, arboviruses may have multiple life cycles, since the physical morphology of these virions may be a mosaic form *in vivo* [62]. These features may be one of the reasons why developing a vaccine against arboviruses is such a difficult task. Despite the potential dilemma, there are some successes; though continued improvement in developing arboviral vaccines that are capable of preventing encephalitis is an urgently needed and challenging task.

Other foreseeable methods for areas where arboviral encephalitis is prevalent include insecticide spraying, which may be used to control outbreaks. Wearing insect repellent and avoiding outdoor activities when mosquitoes are active may also be helpful.

9. Conclusion and perspective

Arboviral encephalitis is a very significant human disease and is caused by a large group of viruses distributed across multiple virus families. The virus is introduced to human beings by hematophagous arthropods, mainly mosquitoes and ticks. With a wide spectrum of clinical manifestations, the diseases are very difficult to diagnose and treat. Although arboviral infections are vaccine preventable and treatable diseases, only a couple of anti-viral thera-

peutic drugs and vaccines are available. However, several new drugs are undergoing clinical trials and some will likely become available within 5 years. Animal models that can capture the cardinal features of disease seen in their human counterparts will be a very critical technological advance. Using adequate animal models can pave the way for understanding and uncovering the paramount host and viral factors responsible for breaking down the BBB and leading to the penetration of the virus into the CNS, as well as serving as a good platform to test for effective and preventive modalities.

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