

Review

Zika virus: epidemiology, clinical features and host-virus interactions

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Abstract

Very recently, Zika virus (ZIKV) has gained a medical importance following the large-scale epidemics in South Pacific and Latin America. This paper reviews information on the epidemiology and clinical features of Zika disease with a particular emphasis on the host-virus interactions that contribute to the pathogenicity of ZIKV in humans.

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1. Introduction

Zika virus (ZIKV) is a little known arbovirus that was initially identified in Uganda in 1947 in a rhesus monkey used as a sentinel during sylvatic yellow fever surveillance in the Zika forest in Uganda [1]. The virus was first reported in humans in Uganda and in Tanzania in 1952 [2]. Before 2007, ZIKV was reported as causing only sporadic human infections in tropical Africa and in some areas in Southeast Asia. Since 2007, several outbreaks have been documented across the Pacific Islands showing the viral circulation outside its previously known geographic region. Autochthonous transmission of ZIKV in South America was reported in early 2015 [3,4]. Since the first report of Zika fever on the African continent until mid-2000, few scientific articles, mainly reporting human cases and describing potential vectors of ZIKV, have been published and, due to the absence of epidemic episodes, as well as serious clinical consequences of viral infection, the interest of the scientific community remained low. However, in

2007 the first large outbreak occurred on the island of Yap, in Micronesia, resulting in a drastic change in the outlook on the ZIKV infection and a growing interest for this newcomer to the world of arboviruses (Fig. 1). Until recently, the clinical manifestations of ZIKV infection ranged from asymptomatic infections to mild, self-limited febrile illness, similar to that of a mild dengue-like syndrome, characterized by fever, headache, muscle and joint pains, as well as a characteristic maculopapular rash reminiscent to measles. Moreover, the disease occurred mainly within a narrow equatorial belt from Africa to Asia. However, an association with neurological complications such as Guillain-Barre Syndrome and congenital microcephaly has been recently suspected [5,6], in particular following spreading of the virus in the Americas where the vectors are present. Given the rapid worldwide spread of ZIKV and the current pandemic in Latin America and the Caribbean, it is now considered as an emerging infectious disease.

To date, the biology of ZIKV infection remains still poorly understood, as compared with other emerging arboviruses such as Yellow fever, Dengue (DENV), West Nile (WNV), Japanese encephalitis (JEV) and Chikungunya (CHIKV)

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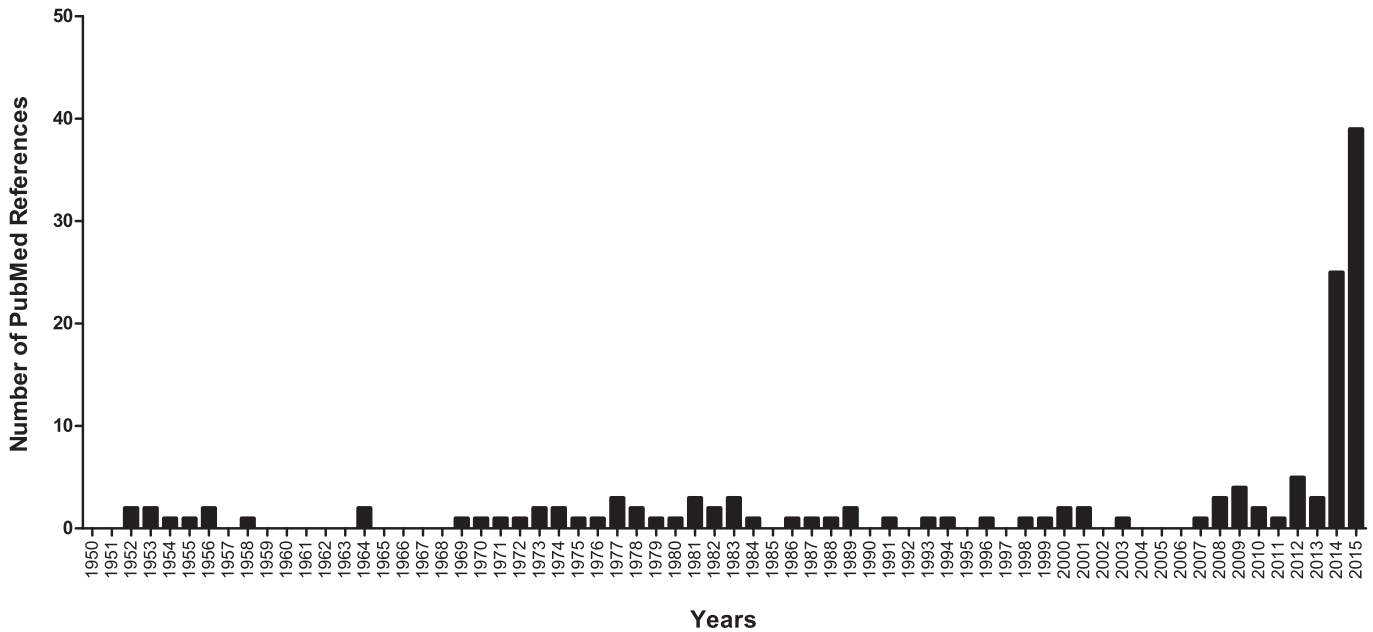


Fig. 1. **Publication related to ZIKV in PubMed database.** Articles published between 1950 and January 2016. Recording of publication was done using the term “Zika virus” and are reported by 10-year periods.

viruses. Here, we review recent progress on the epidemiology and the cell biology of infection by ZIKV.

2. Zika virus

2.1. Zika lineages

The zoonotic ZIKV is a single-stranded positive RNA virus belonging to the *flavivirus* genus of *Flaviviridae* family. The medically important mosquito-borne flaviviruses are DENV, WNV, JEV, yellow fever virus, tick-borne encephalitis and now ZIKV [7]. Phylogenetic analysis and genomic comparison of ZIKV have revealed two major lineages, Asian and African [8,9] (Fig. 2 A, B and C). The finding that the African lineage is subdivided in two clades suggesting two different introductions [7].

Faye et al. proposed the geographical spreading of ZIKV from the MR766 original Uganda strain and referred to two independent ZIKV introductions from East to West Africa [7]. In addition, it is possible that the Malaysian strain has originated from Uganda ZIKV strain in the middle of the 20th century and that the Asian lineage originates from this initial Malaysian strain.

Serological evidence of ZIKV circulation in Borneo, India, Indonesia, Malaysia, Thailand and Vietnam was reported in the early 1950's [8]. According to Faye et al., whether the Asian lineage originated from an African strain the question arises of the rapid spread in Asia. However, other hypotheses could be taken into account: i/ZIKV was introduced in Asia in the middle of 20th, ii/both ZIKV lineages have had a parallel evolution from an ancient common ancestor.

The few ZIKV strains sequenced thus far has limited phylogenetic investigation. Little is know about the

relationships between African and Asian lineages and further retrospective investigation have to be done to improve the knowledge on the genetic history of ZIKV. A deep analysis of the differences between the two lineages as a result of possible mutations could help to understand the observed neuronal complications. In addition, the question concerning the natural host of the virus remains unresolved. Some studies highlight serological evidence in monkeys and other mammals [10]. Nonetheless except in two cercopithecus monkey species, no ZIKV have been successfully isolated from African and Asian wild life [8].

3. Epidemiology

3.1. Vector

After the first isolation of ZIKV from *Aedes (Ae.) africanus* in 1948, it was detected, mainly in sylvatic, *Aedes* genus mosquitoes, including *Aedes furcifer*, *luteocephalus*, *vitattus* and. *apicoargenteus* [1,8,11]. In several countries, ZIKV has been isolated from the antropophilic *Aedes aegypti* mosquitoes [12], the major vector of DENV and CHIKV around the world [13], which is considered to be the main vector of the ZIKV in South and South-East Asia [12]. During the two major outbreaks in the Pacific Islands, *Aedes hensilli* and *Aedes polynesiensis* were also identified as potential vectors of ZIKV [14]. More importantly, however, another major DENV and CHIKV vector *Aedes albopictus*, was recently found to be infected by ZIKV [15]. The competence of *Ae. aegypti* and *Ae. albopictus* to transmit ZIKV has been demonstrated in two studies carried out at an interval of nearly 50 years [16]. The results of both studies pointed to a short incubation period, ranging from 7 to 10 days, during which the virus replicates in

the mosquitoes' midgut, and then spreads toward the salivary glands which is associated with high levels of transmission. Nevertheless, the superiority of either vector over the other in its capacity to optimally transmit ZIKV has yet to be determined.

Although the virus reservoir is not yet clearly identified, some authors have suggested that ZIKV might be maintained in nature either by a sylvatic cycle, involving non-human primates, or in a broad range of *Aedes* mosquito's species [17,18]. In contrast, in urban areas, the transmission would be assured by the anthropophilic mosquitoes *Ae. aegypti* and *Ae. albopictus* [12,18]. The latter one is particularly worrisome because of its diurnal feeding and their habit to bite multiple hosts during the development cycle of their eggs which makes them very efficient at transmitting diseases. However, serological studies have demonstrated the presence of specific antiviral antibodies in various mammals, among which rodents, elephants and felines, which suggests that other reservoirs may play a role in the transmission cycle of the virus [8,10]. It should be noted that non-human primate populations are absent in many Pacific islands where the virus has been detected and the nature of the viral reservoir in this area therefore remains speculative. There is an urgent need to identify vectors and potential vectors of ZIKV in vulnerable area in order to control disease outbreaks.

3.2. Geographic distribution

Following the first isolation of ZIKV from a human in Uganda in 1952 [19], sporadic cases of human infection were detected in Africa [20,21] and later in South East Asia [22] (Fig. 3). Subsequently, serological, virological and entomological studies on non-human primates, human and mosquitoes pointed to a more extensive distribution in Africa, in particular in Gabon, Ivory Cost, Burkina Faso, Sierra Leone, Cameroun, Ethiopia, Kenya, Tanzania, Somalia and Egypt, as well as in Asian countries, including Malaysia, India, the Philippines, Thailand, Vietnam, Indonesia, and Pakistan [10,19]. Although ZIKV was discovered more than 60 years ago, only 14 human cases were reported before 2007 after which it emerged during four major outbreaks in Yap Island, French Polynesia, New Caledonia and, very recently, in Brazil (Fig. 3).

The first marked outbreak was reported on Yap Island, Federated States of Micronesia, in the Pacific Ocean [14], a little island with approximately 7000 residents. Results from a seroprevalence study estimated that 73% of the population was infected with ZIKV within 4 months, with about 80% of asymptomatic patients [23], highlighting for the first time the infectious capacities of the virus and marking the starting point of its spreading across the Pacific area.

During the winter of 2013–2014, a wider outbreak developed in French Polynesian Islands affecting about 1% of the population [9]. Phylogenetic analysis of partial membrane/envelop genes showed the close relationship of the French Polynesian strain to those emerging in Cambodia in 2010 and Yap Island in 2007, thus highlighting the spreading of the

ZIKV Asian lineage [7,8]. In the sylvatic transmission cycle, humans are considered accidental hosts. In contrast, during the outbreaks in Yap Island and French Polynesia where non human monkeys are lacking, human beings were considered as the primary amplifying host of ZIKV, as already observed for other arboviruses such as CHIKV and DENV [13,14].

In 2014 and 2015, following the introduction of a ZIKV belonging to the Asian lineage in French Polynesia, an outbreak occurred in New Caledonia with about 1500 cases [24]. During the same year, the presence of ZIKV was confirmed in Eastern Island [25] whereas imported ZIKV cases were documented in non-endemic areas including Japan [26], Canada [27], Australia [28] and Italy [29].

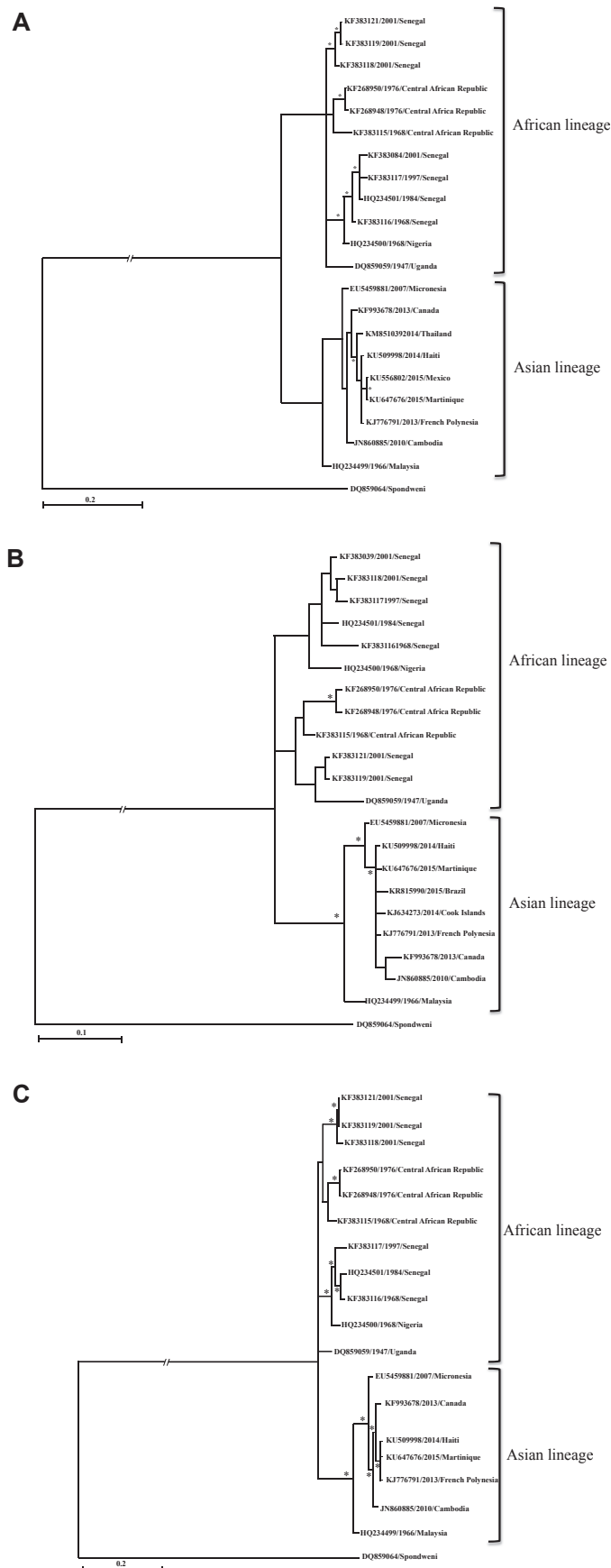
In May 2015, the Brazilian Health authority reported an autochthonous presence of ZIKV in the states of Bahia and Rio Grande de Norte [3] marking the first emergence of ZIKV in the Americas. Phylogenetic analysis of the ZIKV sequences showed that the virus belonged to the Asian lineage and was closely related to the strain isolated during the outbreak on French Polynesia. As of December 2015, several Latin-American countries, including Paraguay, Venezuela, Colombia, Suriname, Panama, El Salvador, Guatemala and Mexico have reported autochthonous cases of ZIKV.

In the beginning of January 2016, more than 150 cases of human infection by ZIKV were detected in Martinique Island by the regional health agency (ARS). This most recent information demonstrates the extraordinary capacity of ZIKV to rapidly spread to non-endemic areas throughout the world where the mosquito vector is present.

3.3. Transmission

Viral transmission occurs during the blood feeding of *Ae.* mosquitoes. Even if mosquito bites are the main mode of transmission, some cases of non-vector-borne infection have also been reported, referred to as perinatal transmission [30], probably following viral crossing of the placenta or during the delivery by viraemic mothers, with mother and baby presenting the same clinical signs of the disease. Although the virus has been detected in breast milk, no evidence exists as yet about the possible transmission via this route. It is of note that before spreading of ZIKV in the Americas, infection of newborns did not result in notable clinical manifestations, other than those observed in adults. Transmission by blood transfusion has not yet been demonstrated although a potential risk cannot be excluded [31]. During the French Polynesia outbreak, the prevalence in blood samples was 1.9%, whereas 74% were found to be asymptomatic. However, the viral load in patients during the incubation period or in asymptomatic people has not been determined. The viral genome has been detected in saliva [32] and urine [33]. This finding is of interest because the presence of viral RNA was revealed between two to three weeks after the onset of clinical symptoms, whereas, at that time, it was not yet detectable in blood samples.

Finally, one sexual transmission was reported in the USA [34] and the presence of ZIKV was demonstrated in the semen



of an infected patient in French Polynesia [35]. The latter transmission route has not been reported before and is rare in arboviral infection: these findings nevertheless support the hypothesis that sexual relations could potentially transmit ZIKV (<http://www.cdc.gov/zika/transmission/sexual-transmission.html>).

4. Clinical features

4.1. Symptomatology

Asymptomatic patients are frequent, reaching up to 80%, and they constitute a high-risk source of transmission [36]. The incubation period ranges from 3 to 12 days, followed by a mild “dengue-like” syndrome for a period of 2–7 days with a broad range of symptoms, including the presence of huge maculopapular rashes, a state of mild fever and headaches, arthralgia, retro-orbital pain, conjunctivitis and edema of the extremities [14,19,22]. The eruption of maculopapular rashes presented by more than 90% of patients remains the main clinical symptom that characterizes ZIKV infection [37].

While the majority of human cases were benign, during the French Polynesian epidemic several neurological complications were reported presenting Guillain-Barré Syndrome (GBS), an autoimmune disease, due to damage to the peripheral nervous system with a loss of the myelin insulation resulting in myalgia, facial palsy and muscle dysfunction. During the French Polynesian outbreak, a patient who presented with GBS was diagnosed with Zika fever. Following this first case, about 72 cases of GBS were reported with 40 patients being seropositive for the presence of the virus and link with ZIKV infection was put forward. The incidence resulted in an unexpected increase of GBS by 20 fold [5]. However, the direct relationship between the virus and GBS need to be confirmed because of co-circulation of DENV (serotype 1 and 3) and ZIKV during this outbreak. Recently, the peculiarity of the ZIKV outbreak in Brazil has shown for the first time a possible link between ZIKV infection in pregnancy and microcephaly of the fetus [6]. Congenital microcephaly is characterized by a fetal head circumference under the average for gestational age with the most common resulting disability being intellectual retardation and physical disability. The incidence of congenital microcephaly in Brazil has increased dramatically from approximately 150 cases per year between 2010 and 2015 to almost double that during the first 9 months of 2015. Since then, cases have shot up to over

Fig. 2. Phylogenetic analysis of the isolated strains ZIKV. Non-concatenated nucleic acid sequences of the NS5 (A) and partial E (B) genes and full length genomes (C) from sequences available on GeneBank data bases were aligned using MEGA 5 [71], with minor manual adjustments. Sites that could not be unambiguously aligned were excluded and divergent regions were excluded from subsequent analyses. Phylogenetic trees were inferred using Bayesian method implemented in Topali software. Mr Bayes ran for 500 000 generations for NS5 and partial E respectively, and with 1.000.000 generations for the complete genomes with a 10% burn-in. Spondweni virus strain SM-6 V-1 (GenBank accession number DQ859064) was used as out-group for all phylogenies.

2000 in just a few months. Transplacental ability of ZIKV has been demonstrated by the presence of viral RNA in the amniotic fluid of pregnant women with fetal microcephaly [6]. In addition to microcephaly, the possible relation between ZIKV infection and hydrops fetalis and fetal demise has recently reported in the same region [38]. All these data suggest a possible materno-fetal transmission. Although maternal–fetal transmission has been already described in DENV and WNV, no other flavivirus is known to have teratogenic effects. Nevertheless, the microcephaly epidemic in Brazil could also be linked to any other cause, such as other infectious or environmental agents. A recent study has revealed some ocular manifestations in three infants with microcephaly with one presenting with a macular neuroretinal atrophy [39]. However, further studies are needed to better define the outcomes of ZIKV infection during pregnancy. In this respect, although there is clear evidence of an increased number of cases of microcephaly in Brazil, it has been suggested that the number of suspected cases might be overestimated because of the diagnosis relying on low-specificity screening tests and the inclusion of mostly normal children with small heads [40] requiring a stricter application of standardized anthropometric techniques and confirmation of suspected cases by laboratory or radiological evidence. There are no specific treatments or vaccine available against ZIKV and the treatment remained only a symptomatic support. The main means to combat infection are based on vector control and bite prevention.

4.2. ZIKV detection and laboratory diagnosis

Zika diagnosis remains difficult due to the similar clinical presentation of other arbovirus infections like dengue or chikungunya. Furthermore, DENV and CHIKV co-circulate in the same geographical areas, very likely resulting in an underestimation of human cases during periods of co-epidemics [41]. The differential diagnosis is challenging and often patients are diagnosed initially as having DENV infection. Before the contribution of molecular biology, the diagnostic assays for ZIKV were based on virus isolation and serological methods. Although these techniques remain currently relevant, the isolation of the virus is time consuming, requiring samples from convalescent patients or those with an acute infection, whereas cross-reactions with related flaviviruses is still a major issue. Serum antibody specificity can be determined using viral neutralizing assays which require highly specialized medical laboratories and remain largely complicated in their interpretation. Moreover, the viremic period is short (up to 5 days after disease onset) which complicates the detection of antibodies [23]. At present, techniques permitting serological detection by immunofluorescence or ELISA are still under development and not commercially available. To improve the diagnostics, alternative molecular biology methods were developed for a wide range of flaviviruses [42] including ZIKV [43]. These methods are rapid, sensitive and specific, but have to be used at the onset of the disease. Specific molecular assays, targeting the NS5 region or the envelope gene

for the Asian and African ZIKV strains have been published [7,23]. With respect to serological methods, commercial detection test do not exist. It would be of interest to develop a diagnostic method using urine samples because, during the French Polynesia outbreak, Zika virus genome RNA was detected in this corporal fluid up to 10 days after onset of disease in several patients [33].

5. The pathogenicity of ZIKV in humans

5.1. ZIKV genomic organization and replication

Characteristic of the Flavivirus genus, ZIKV has a ~11 kb positive strand RNA genome [44] which is composed of a single open reading frame, 10,794 pb in length, flanked by two non-coding regions (Fig. 4). The genome is translated into a single large polyprotein that is subsequently cleaved into three structural proteins, envelope (E), membrane precursor (prM) and capsid (C), as well as seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5), necessary for viral replication and assembly [42]. The envelope protein is involved in the recognition of the receptor at the surface of the host cell and then the fusion process between the viral envelope and the intracellular membranes [45]. The different stages of ZIKV replication have not yet been well defined, but it is thought that they are essentially similar to other members of *flavivirus* genus. Typically, the virus has to complete four major stages to finalize its replicative cycle: translation of genomic RNA into viral proteins, replication of viral RNA molecules, assembly of virus particles in the endoplasmic reticulum and the release of virions [46]. The virion has an icosahedral capsid enclosed by a lipid envelope with a diameter of 40–70 nm [47]. ZIKV replication occurs in the cellular cytoplasm and, like all other flaviviruses, multiple relationships with cellular organelles might exist to facilitate viral replication, evasion and propagation, although the exact mechanisms remain to be determined. The identification of such host factors and the characterization of their interactions with viral RNA and proteins are important for the understanding of ZIKV replication. Comprehensive analysis of the 5' and 3' untranslated regions of ZIKV also need to be carried out as these RNA structures play a critical role in viral genome cyclization and replication.

5.2. ZIKA virus permissiveness of human cells

Arthropod-mediated transmission of arbovirus is initiated when a blood-feeding female injects the virus into the human skin (reviewed in Ref. [48]). Like many other members of the flavivirus family, ZIKV is transmitted following the bite of *Aedes* mosquitoes [15,16]. Different cells types, such as epidermal keratinocytes [49], dendritic cells [50] or neurons [51] are known to be a target of flaviviruses. Given the knowledge on the entry route of flaviviruses, potential target cells for infection with ZIKV have been investigated. Recently, we have reported that various cells in the human skin compartment are able to support ZIKV replication [47].

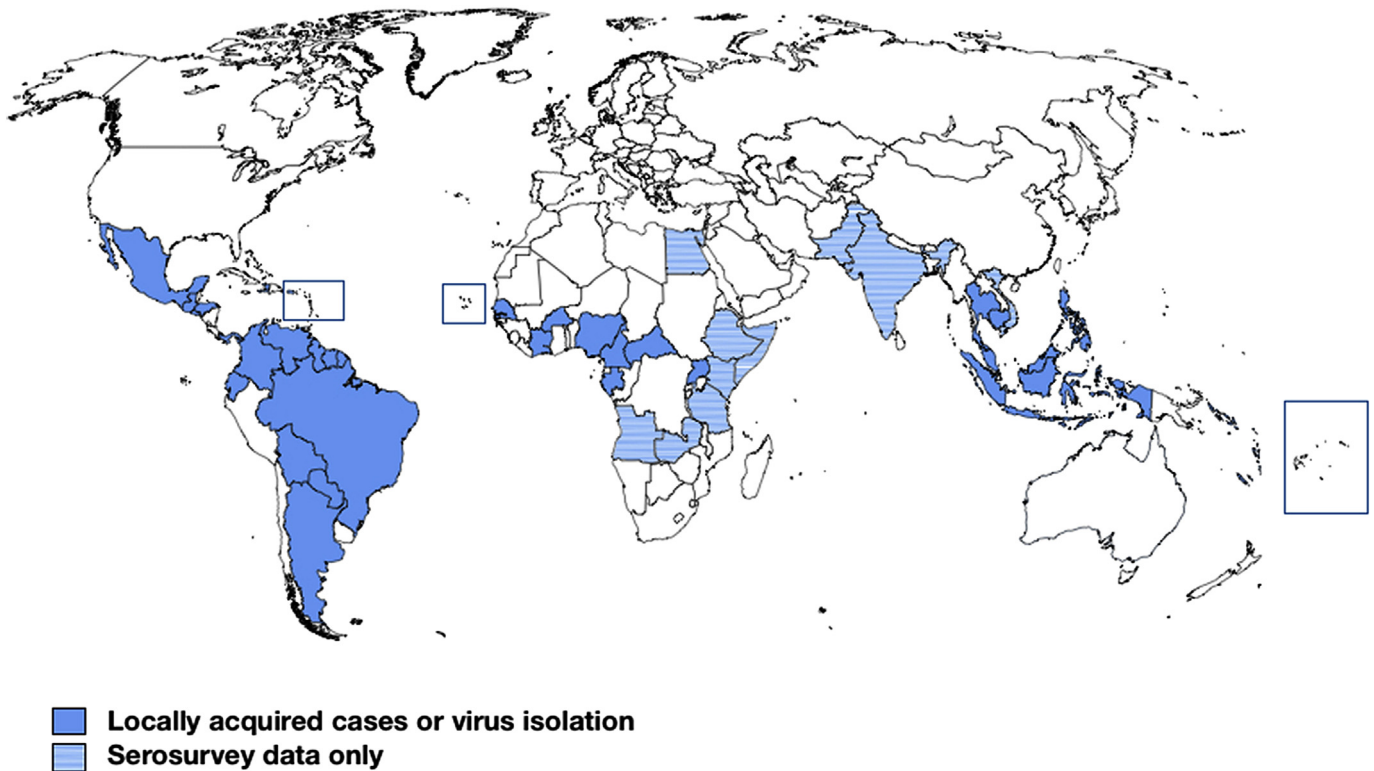


Fig. 3. **Geographic distribution of ZIKV.** Adapted from Centers for Disease Control and Prevention publication, <http://www.cdc.gov/zika/geo/index.html>.

Following infection with ZIKV, viral replication was observed in fibroblasts, keratinocytes and immature dendritic cells (iDCs), in a time dependent manner, with a substantial percentage of infected cells as early as 24 h post infection, whereas all cells were able to produce infectious virions. These results provide strong evidence for the critical role of the skin compartment in the transmission of ZIKV and are in line with those reported previously with respect to other members of the flavivirus genus [52].

The interaction between the E glycoprotein of the viral particle and cell surface receptors allows the entry of the flaviviruses into the target cells. However, despite many investigations, the key cellular receptors remain relatively unknown and their importance in viral entry have yet to be clearly established. Several of those receptors have already been reported to permit viral entry of DENV and others arboviruses [53]. DC-SIGN which is highly expressed on iDCs and macrophages [54] has been known for many years to permit attachment and infection by Dengue virus [55,56] thereby facilitating viral dissemination.

The TIM family is constituted of three receptors, TIM-1, TIM-3 and TIM-4. TIM-1 is expressed by Th2 cells and epithelial cells, whereas Th1 cells essentially express TIM-3. The expression of TIM-4 is restricted to antigen presenting cells [53]. TIMs receptors have different roles, such as phosphatidylserine (PtdSer)-dependent phagocytic removal of apoptotic cells, or regulation of innate and adaptive immune responses. TIM-1 and TIM-4 expression is highly regulated following infection with DENV, WNV or YFV. The

importance of TIM-1 receptor in infection was demonstrated using silencing technologies or blocking antibodies [57].

The TAM receptor family is composed of TYRO3, AXL and MER receptors that are protein tyrosine kinases contributing to the regulation of immune responses [58]. Whereas AXL and MER are widely expressed, TYRO3 expression is mainly observed in the central nervous system. TAM receptors have been recently described to mediate DENV, WNV and YFV entry, both in cell lines and primary human cells [57].

It was recently shown that DC-SIGN, TYRO3, and especially AXL, play an important role in the entry and replication of ZIKV in human skin cells [47]. Specific antibodies or the use of siRNA interference to silence receptor strongly inhibited ZIKV replication. It is important to note that, in contrast to what has been reported for DENV infection, TIM-1 and TIM-4 receptors seem to play a minor role in the entry of ZIKV in human skin cells, although a cooperative role of both TIM and TAM family members has been observed. The exact contribution of each of these receptors to ZIKV entry is still under investigation.

5.3. Innate immune response to ZIKV

Following viral infection, cells mount a range of antiviral responses in order to limit the spread of the virus, with the main defense consisting of innate and adaptive immune responses. The initial response is provided for by the production of type I interferons (IFNs) [59]. Early detection of pathogen-associated molecular patterns (PAMP) expressed

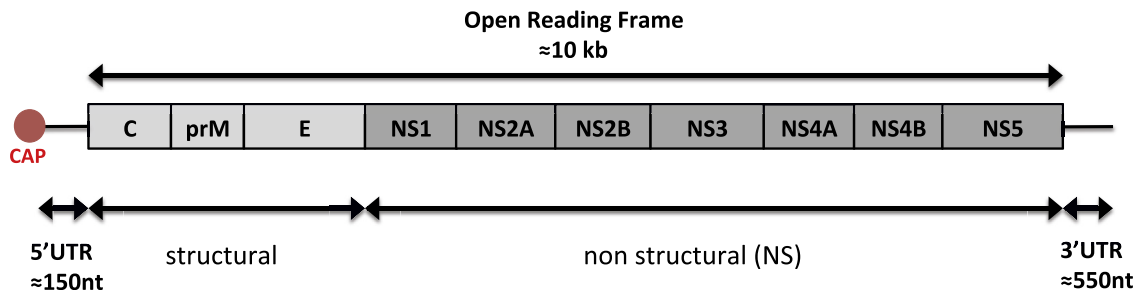


Fig. 4. **Organization of the ZIKV genome.** The genomic organization is arranged as with other flaviviruses, with an open reading frame encoding three structural proteins C, prM and E and five nonstructural proteins NS1 to NS5. The structural and nonstructural proteins are generated after proteolytic cleavage of the whole translated polypeptide from the ORF region.

by the virus is sensed and mediated by pattern recognition receptors (PRRs), such as the Toll-like receptor (TLR)-family or RIG-I like receptor. Following the detection of PAMPs, the triggering of various signaling pathways not only leads to a direct secretion of IFNs, but also the expression of hundreds of Interferon-Stimulated Genes (ISGs) that integrate to induce an antiviral state of cells [60]. DENV and WNV infections have been the subject of a large body of studies that show a pivotal role for RIG-I, MDA5 and TLR3 sensors in the host defense against these viruses, leading to IFN type 1 production and expression of ISGs [61]. In a similar fashion, infection of human fibroblasts with ZIKV induces the expression of RIG-I, MDA-5 and TLR3, as well as the ISGs ISG15, OAS2 and MX1. Moreover, increased expression levels of IRF7, a transcription factor that binds to promoters on IFN type I genes, corroborates the strong induction of IFN- α and IFN- β by ZIKV-infected fibroblasts. Furthermore, the expression of certain inflammatory chemokines, such as CCL5, is also induced upon ZIKV infection. The inflammatory pathway seems to be activated as well following ZIKV infection, as shown by an increase in the expression of AIM2 and interleukin-1 β transcripts [47]. These results notwithstanding, the involvement of the latter pathway remains to be investigated in more detail in order to better understand the involvement of interleukin-1 β in the inhibition of ZIKV replication.

Different cellular processes can be hijacked by flaviviruses to evade the cellular response and facilitate virus replication. Immediately following infection, the host establishes rapid innate immune responses, including IFN type I responses, the induction of apoptosis and autophagy, in order to overcome the viral infection review in Ref. [62]. WNV is able to elude detection or inhibit IFN gene transcription [63,64]. In addition, several arboviruses such as DENV and CHIKV can subvert the autophagy process [65,66] in order to promote their own replication and dissemination. It is known that flaviviruses rearrange host cell membranes to create an appropriate environment for their replication with the main source of membranes being the endoplasmic reticulum [67]. These rearrangements result in an activation of the unfolded protein response (UPR) and overexpress the autophagic pathway in infected cells simultaneously [68]. The double-membrane vesicles, known as autophagosome, allow the recruitment of

cytoplasmic elements, proteins and organelles and permit their degradation. Autophagy could act both positively or negatively in host immunity against pathogens [69,70]. In the last mechanism, viruses use the autophagy pathway to facilitate their own replication.

The first evidences of autophagy in ZIKV-infected fibroblasts, were highlighted by the presence of characteristic autophagosome-like vesicles in infected fibroblasts [47]. Then, the authors confirmed the induction of autophagy in infected fibroblasts by an increase in production of LC3, a cytosolic microtubule-associated molecule. The latter was colocalized with viral envelope protein suggesting that autophagocytic vacuoles are the site of viral replication. The use of an inhibitor of the autophagosome formation during the infection significantly reduces viral copy number while an inducer of autophagy increases viral replication, demonstrating that autophagy could play a major role in ZIKV immune evasion. However, the intimate molecular mechanisms remain to be further investigated.

6. Conclusion

The most recent ZIKV outbreaks and in particular its introduction in the Americas have resulted in a change in the appreciation of this virus and the clinical repercussions of its presence in the human host. Like other arboviruses, the rapid spreading of ZIKV is due to favorable climatic and socio-demographic conditions, including the presence of its mosquito vectors in urban concentrations with poor hygienic conditions, the increase in international travel and possibly a better adaptation of the mosquitoes to humans. This situation is extremely worrisome and requires efficient surveillance systems, including sensitive diagnostic methods and vector control measures. The co-circulation of ZIKV with other medically important arboviruses, such as DENV and CHIKV, constitutes an additional challenge which complicates the comprehension of this disease. The suspected association between Zika and neurological complications such as GBS and congenital malformation needs to be confirmed in order to control and reduce the negative impacts of the infection. A thorough understanding of the molecular interactions that ZIKV establishes with the host cell during infection is also necessary to determine the targets for antiviral treatment.

Conflict of interest

We the authors declare no conflict of interest in writing this review.

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References

- [1] Dick GW, Kitchen SF, Haddock AJ. Zika virus. I. Isolations and serological specificity. *Trans R Soc Trop Med Hyg* 1952;46:509–20.
- [2] Dick GW. Zika virus. II. Pathogenicity and physical properties. *Trans R Soc Trop Med Hyg* 1952;46:521–34.
- [3] Campos GS, Bandeira AC, Sardi SI. Zika virus outbreak, Bahia, Brazil. *Emerg Infect Dis* 2015;21:1885–6.
- [4] Zanusca C, de Melo VC, Mosimann AL, Dos Santos GI, Dos Santos CN, Luz K. First report of autochthonous transmission of Zika virus in Brazil. *Mem Inst Oswaldo Cruz* 2015;110:569–72.
- [5] Oehler E, Watrin L, Larre P, Leparc-Goffart I, Lastere S, Valour F, et al. Zika virus infection complicated by Guillain-Barre syndrome - case report, French Polynesia. *Euro Surveill* December 2013;2014;19. pii 20720.
- [6] Oliveira Melo AS, Malinger G, Ximenes R, Szejnfeld PO, Alves Sampaio S, Bispo de Filippis AM. Zika virus intrauterine infection causes fetal brain abnormality and microcephaly: tip of the iceberg? *Ultrasound Obstet Gynecol* 2016;47:6–7.
- [7] Faye O, Freire CC, Iamarino A, de Oliveira JV, Diallo M, Zanotto PM, et al. Molecular evolution of Zika virus during its emergence in the 20(th) century. *PLoS Negl Trop Dis* 2014;8:e2636.
- [8] Haddock AD, Schuh AJ, Yasuda CY, Kasper MR, Heang V, Huy R, et al. Genetic characterization of Zika virus strains: geographic expansion of the Asian lineage. *PLoS Negl Trop Dis* 2012;6:e1477.
- [9] Cao-Lormeau VM, Roche C, Teissier A, Robin E, Berry AL, Mallet HP, et al. Zika virus, French Polynesia, South Pacific. *Emerg Infect Dis* 2013;2014(20):1084–6.
- [10] Darwish MA, Hoogstraal H, Roberts TJ, Ghazi R, Amer T. A sero-epidemiological survey for Bunyaviridae and certain other arboviruses in Pakistan. *Trans R Soc Trop Med Hyg* 1983;77:446–50.
- [11] Hayes EB. Zika virus outside Africa. *Emerg Infect Dis* 2009;15:1347–50.
- [12] Li MI, Wong PS, Ng LC, Tan CH. Oral susceptibility of Singapore *Aedes (Stegomyia) aegypti* (Linnaeus) to zZika virus. *PLoS Negl Trop Dis* 2012;6:e1792.
- [13] Diallo M, Thonnon J, Traore-Lamizana M, Fontenille D. Vectors of Chikungunya virus in Senegal: current data and transmission cycles. *Am J Trop Med Hyg* 1999;60:281–6.
- [14] Duffy MR, Chen TH, Hancock WT, Powers AM, Kool JL, Lanciotti RS. Zika virus outbreak on Yap Island, Federated States of Micronesia. *N Engl J Med* 2009;360:2536–43.
- [15] Grard G, Caron M, Mombou IM, Nkoghe D, Mboui Ondo S, Jiolle D, et al. Zika virus in Gabon (Central Africa) - 2007: a new threat from *Aedes albopictus*? *PLoS Negl Trop Dis* 2014;8:e2681.
- [16] Wong PS, Li MZ, Chong CS, Ng LC, Tan CH. *Aedes (Stegomyia) albopictus* (Skuse): a potential vector of Zika virus in Singapore. *PLoS Negl Trop Dis* 2013;7:e2348.
- [17] Grard G, Moureau G, Charrel RN, Holmes EC, Gould EA, de Lamballerie X. Genomics and evolution of *Aedes-borne flaviviruses*. *J Gen Virol* 2010;91:87–94.
- [18] Wolfe ND, Kilbourn AM, Karesh WB, Rahman HA, Bosi EJ, Cropp BC, et al. Sylvatic transmission of arboviruses among Bornean orangutans. *Am J Trop Med Hyg* 2001;64:310–6.
- [19] Simpson DI. Zika virus infection in man. *Trans R Soc Trop Med Hyg* 1964;58:335–8.
- [20] Macnamara FN. Zika virus: a report on three cases of human infection during an epidemic of jaundice in Nigeria. *Trans R Soc Trop Med Hyg* 1954;48:139–45.
- [21] Monlun E, Zeller H, Le Guenno B, Traoré-Lamizana M, Hervy JP, Adam F, et al. Surveillance of the circulation of arbovirus of medical interest in the region of eastern Senegal. *Bull Soc Pathol Exot* 1993;86:21–8.
- [22] Heang V, Yasuda CY, Sovann L, Haddock AD, Travassos da Rosa AP, Tesh RB, et al. Zika virus infection, Cambodia. *Emerg Infect Dis* 2010;2012(18):349–51.
- [23] Lanciotti RS, Kosoy OL, Laven JJ, Velez JO, Lambert AJ, Johnson AJ, et al. Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia. *Emerg Infect Dis* 2007;2008(14):1232–9.
- [24] Dupont-Rouzeyrol M, O'Connor O, Calvez E, Daurès M, John M, Grangeon JP, et al. Co-infection with Zika and dengue viruses in 2 patients, New Caledonia. *Emerg Infect Dis* 2014;2015(21):381–2.
- [25] Tognarelli J, Ulloa S, Villagra E, Lagos J, Aguayo C, Fauce R, et al. A report on the outbreak of Zika virus on Easter Island, South Pacific, 2014. *Arch Virol* 2016;161:665–8.
- [26] Kutsuna S, Kato Y, Takasaki T, Moi M, Kotaki A, Uemura H, et al. Two cases of Zika fever imported from French Polynesia to Japan, December 2013 to January 2014. *Euro Surveill* 2014;19. pii 20683.
- [27] Fonseca K, Meatherall B, Zarra D, Drebot M, MacDonald J, Pabbaraju K, et al. First case of Zika virus infection in a returning Canadian traveler. *Am J Trop Med Hyg* 2014;91:1035–8.
- [28] Pyke AT, Daly MT, Cameron JN, Moore PR, Taylor CT, Hewitson GR, et al. Imported Zika virus infection from the Cook islands into Australia, 2014. *PLoS Curr* 2014;6.
- [29] Zammarchi L, Stella G, Mantella A, Bartolozzi D, Tappe D, Gunther S, et al. Zika virus infections imported to Italy: clinical, immunological and virological findings, and public health implications. *J Clin Virol* 2015;63:32–5.
- [30] Besnard M, Lastere S, Teissier A, Cao-Lormeau V, Musso D. Evidence of perinatal transmission of Zika virus, French Polynesia, December 2013 and February 2014. *Euro Surveill* 2014;19. pii 20751.
- [31] Musso D, Nhan T, Robin E, Roche C, Bierlaire D, Zisou K, et al. Potential for Zika virus transmission through blood transfusion demonstrated during an outbreak in French Polynesia, November 2013 to February 2014. *Euro Surveill* 2014;19. pii 20771.
- [32] Musso D, Roche C, Nhan TX, Robin E, Teissier A, Cao-Lormeau VM. Detection of Zika virus in saliva. *J Clin Virol* 2015;68:53–5.
- [33] Gourinat AC, O'Connor O, Calvez E, Goarant C, Dupont-Rouzeyrol M. Detection of Zika virus in urine. *Emerg Infect Dis* 2015;21:84–6.
- [34] Foy BD, Kobylinski KC, Chilson Foy JL, Blitvich BJ, Travassos da Rosa A, Haddock AD, et al. Probable non-vector-borne transmission of Zika virus, Colorado, USA. *Emerg Infect Dis* 2011;17:880–2.
- [35] Musso D, Roche C, Robin E, Nhan T, Teissier A, Cao-Lormeau VM. Potential sexual transmission of Zika virus. *Emerg Infect Dis* 2015;21:359–61.
- [36] Musso D, Nilles EJ, Cao-Lormeau VM. Rapid spread of emerging Zika virus in the Pacific area. *Clin Microbiol Infect* 2014;20:O595–6.
- [37] Mallet HP, Vial AL, Musso D. Bilan de l'épidémie à virus Zika en Polynésie française 2013-2014. 2015. p. 1–5.
- [38] Sarno M, Sacramento GA, Khouri R, do Rosario MS, Costa F, Archanjo G, et al. Zika virus infection and stillbirths: a case of hydrops fetalis, hydranencephaly and fetal demise. *PLoS Negl Trop Dis* 2016;10:e0004517.
- [39] Ventura CV, Maia M, Bravo-Filho V, Góis AL, Belfort R. Zika virus in Brazil and macular atrophy in a child with microcephaly. *Lancet* 2016;387:228.
- [40] Villar J, Cheikh Ismail L, Victora CG, Ohuma EO, Bertino E, Altman DG, et al. International standards for newborn weight, length, and head circumference by gestational age and sex: the Newborn Cross-Sectional Study of the INTERGROWTH-21st Project. *Lancet* 2014;384:857–68.

- [41] Roth A, Mercier A, Lepers C, Hoy D, Duituturaga S, Benyon E, et al. Concurrent outbreaks of dengue, chikungunya and Zika virus infections - an unprecedented epidemic wave of mosquito-borne viruses in the Pacific 2012-2014. *Euro Surveill* 2014;19. pii 20929.
- [42] Kuno G, Chang GJ, Tsuchiya KR, Karabatsos N, Cropp CB. Phylogeny of the genus *Flavivirus*. *J Virol* 1998;72:73–83.
- [43] Faye O, Faye O, Dupressoir A, Weidmann M, Ndiaye M. Alpha Sall A. One-step RT-PCR for detection of Zika virus. *J Clin Virol* 2008;43: 96–101.
- [44] Kuno G, Chang GJ. Full-length sequencing and genomic characterization of Bagaza, Kedougou, and Zika viruses. *Arch Virol* 2007;152:687–96.
- [45] Lindenbach BD, Rice CM. Molecular biology of flaviviruses. *Adv Virus Res* 2003;59:23–61.
- [46] Bidet K, Garcia-Blanco MA. Flaviviral RNAs: weapons and targets in the war between virus and host. *Biochem J* 2014;462:215–30.
- [47] Hamel R, Dejarnac O, Wichit S, Ekcharyawat P, Neyret A, Luplertlop N, et al. Biology of Zika virus infection in human skin cells. *J Virol* 2015; 89:8880–96.
- [48] Briant L, Desprès P, Choumet V, Missé D. Role of skin immune cells on the host susceptibility to mosquito-borne viruses. *Virology* 2014; 464–465:26–32.
- [49] Surasombatpattana P, Hamel R, Patramool S, Luplertlop N, Thomas F, Despres P, et al. Dengue virus replication in infected human keratinocytes leads to activation of antiviral innate immune responses. *Infect Genet Evol* 2011;11:1664–73.
- [50] Lozach PY, Burleigh L, Staropoli I, Navarro-Sanchez E, Harriague J, Virelizier JL, et al. Dendritic cell-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN)-mediated enhancement of dengue virus infection is independent of DC-SIGN internalization signals. *J Biol Chem* 2005;280:23698–708.
- [51] Salazar MI, Pérez-García M, Terreros-Tinoco M, Castro-Mussot ME, Diegopérez-Ramírez J, Ramírez-Reyes AG, et al. Dengue virus type 2: protein binding and active replication in human central nervous system cells. *ScientificWorldJournal* 2013;2013:904067.
- [52] Ivory MO, Birchall JC, Piguat V. Early Dengue virus infection in human skin: a cycle of inflammation and infectivity. *J Invest Dermatol* 2015; 135:1711–2.
- [53] Perera-Lecoin M, Meertens L, Carnec X, Amara A. Flavivirus entry receptors: an update. *Viruses* 2014;6:69–88.
- [54] Marovich M, Grouard-Vogel G, Louder M, Eller M, Sun W, Wu SJ, et al. Human dendritic cells as targets of dengue virus infection. *J Invest Dermatol Symp Proc* 2001;6:219–24.
- [55] Navarro-Sanchez E, Altmeyer R, Amara A, Schwartz O, Fieschi F, Virelizier JL, et al. Dendritic-cell-specific ICAM3-grabbing non-integrin is essential for the productive infection of human dendritic cells by mosquito-cell-derived dengue viruses. *EMBO Rep* 2003;4:723–8.
- [56] Lozach PY, Burleigh L, Staropoli I, Navarro-Sanchez E, Harriague J, Virelizier JL, et al. Dendritic cell-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN)-mediated enhancement of dengue virus infection is independent of DC-SIGN internalization signals. *J Biol Chem* 2005;280:23698–708.
- [57] Meertens L, Carnec X, Lecoin MP, Ramdasi R, Guivel-Benhassine F, Lew E, et al. The TIM and TAM families of phosphatidylserine receptors mediate dengue virus entry. *Cell Host Microbe* 2012;12: 544–57.
- [58] Lai C, Lemke G. An extended family of protein-tyrosine kinase genes differentially expressed in the vertebrate nervous system. *Neuron* 1991;6: 691–704.
- [59] Schneider WM, Chevillotte MD, Rice CM. Interferon-stimulated genes: a complex web of host defenses. *Annu Rev Immunol* 2014;32: 513–45.
- [60] Nazmi A, Dutta K, Hazra B, Basu A. Role of pattern recognition receptors in flavivirus infections. *Virus Res* 2014;185:32–40.
- [61] Nasirudeen AM, Wong HH, Thien P, Xu S, Lam KP, Liu DX. RIG-I, MDA5 and TLR3 synergistically play an important role in restriction of dengue virus infection. *PLoS Negl Trop Dis* 2011;5:e926.
- [62] Ye J, Zhu B, Fu ZF, Chen H, Cao S. Immune evasion strategies of *flaviviruses*. *Vaccine* 2013;31:461–71.
- [63] Shipley JG, Vandergaast R, Deng L, Mariuzza RA, Fredericksen BL. Identification of multiple RIG-I-specific pathogen associated molecular patterns within the West Nile virus genome and antigenome. *Virology* 2012;432:232–8.
- [64] Liu WJ, Chen HB, Wang XJ, Huang H, Khromykh AA. Analysis of adaptive mutations in Kunjin virus replicon RNA reveals a novel role for the flavivirus nonstructural protein NS2A in inhibition of beta interferon promoter-driven transcription. *J Virol* 2004;78:12225–35.
- [65] Heaton NS, Randall G. Dengue virus and autophagy. *Viruses* 2011;3: 1332–41.
- [66] Krejbich-Trotot P, Gay B, Li-Pat-Yuen G, Hoarau JJ, Jaffar-Bandjee MC, Briant L, et al. Chikungunya triggers an autophagic process which promotes viral replication. *Virol J* 2011;8:432.
- [67] Blazquez AB, Escribano-Romero E, Merino-Ramos T, Saiz JC, Martín-Acebes MA. Stress responses in flavivirus-infected cells: activation of unfolded protein response and autophagy. *Front Microbiol* 2014;5:266.
- [68] Heaton NS, Randall G. Dengue virus-induced autophagy regulates lipid metabolism. *Cell Host Microbe* 2010;8:422–32.
- [69] Shelly S, Lukinova N, Bambina S, Berman A, Cherry S. Autophagy is an essential component of *Drosophila* immunity against vesicular stomatitis virus. *Immunity* 2009;30:588–98.
- [70] Lee YR, Lei HY, Liu MT, Wang JR, Chen SH, Jiang-Shieh YF, et al. Autophagic machinery activated by dengue virus enhances virus replication. *Virology* 2008;374:240–8.
- [71] Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 2011;28:2731–9.