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Zika Virus

The Agent and Its Biology, With Relevance to Pathology

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• **Once obscure, Zika virus (ZIKV) has attracted significant medical and scientific attention in the past year because of large outbreaks associated with the recent introduction of this virus into the Western hemisphere. In particular, the occurrence of severe congenital infections and cases of Guillain-Barré syndrome has placed this virus squarely in the eyes of clinical and anatomic pathologists. This review article provides a basic introduction to ZIKV, its genetics, its structural characteristics, and its biology. A multidisciplinary effort will be essential to establish clinicopathologic correlations of the basic virology of ZIKV in order to advance development of diagnostics, therapeutics, and vaccines.**

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First identified in 1947 in rhesus macaques in the Zika forest near Kampala, Uganda,[1] Zika virus (ZIKV) received little attention in the medical literature through the 20th century. Few human ZIKV infections were identified, and these were recognized as mild, non–life-threatening febrile illnesses associated with maculopapular pruritic rashes and, less often, with arthralgia or conjunctivitis. Limited seroepidemiologic surveys suggested that as many as 80% of infections were asymptomatic or subclinical.[2]

The global attention to ZIKV has changed drastically in the last decade. In April 2007, an outbreak of illness characterized by rash, arthralgia, and conjunctivitis was reported on Yap Island in the Federated States of Micronesia. Although initial serologic testing suggested that dengue virus (DENV), which had been detected in two previous outbreaks on Yap, might be the causative agent, the illness was clinically distinct from dengue.[2–5] This prompted further testing that identified ZIKV in serum samples. Serosurveys after the outbreak suggested that almost three-quarters of the population had recently been infected with ZIKV. This represented the first recognized outbreak of ZIKV. No deaths or complications were identified.

Outbreaks of ZIKV infection were subsequently recognized in other isolated populations, including French Polynesia in 2013. Ominously, clinicians recognized an increase in the incidence of Guillain-Barré syndrome during the outbreak in French Polynesia,[6–8] the first time such an association was reported. The arrival of ZIKV in the Western hemisphere was heralded by large-scale transmission in Brazil in 2015. In addition to the large number of typical febrile illnesses and an increase in the incidence of Guillain-Barré syndrome, clinicians reported in October 2015 a significant increase in the incidence of microcephaly among newborn infants. Although the association with ZIKV was initially uncertain, evidence accumulated over the course of intense epidemiologic investigations during 2015 convincingly established a causative relationship[9,10] and prompted the World Health Organization to issue a pronouncement of a "Public Health Emergency of International Concern" in February 2016.[11]

The rapid spread of ZIKV through the Americas, leading to millions of cases, the novel associations of ZIKV with congenital microcephaly and Guillain-Barré syndrome, and the recognition of sexual (and perhaps other) mechanisms of ZIKV transmission,[12] have created a rapidly evolving scientific landscape. The explosion of research on ZIKV presents a major challenge, and paradigms derived from knowledge on closely related viruses have proven inadequate. This review attempts to present the fundamental concepts of ZIKV genetics, structure, biology, and host interactions at a cellular level to provide pathologists with key information for interpreting the evolving literature and its significance to clinical and anatomic pathology.

GENETIC AND PHYSICAL STRUCTURE OF ZIKV AND RELATED VIRUSES

Zika virus is classified as a member of the family Flaviviridae. The family Flaviviridae encompasses enveloped viruses with a single-stranded positive sense (same strand as mRNA) RNA genome that encodes all viral proteins from a single open reading frame (Figure 1, A). The polyprotein chain that is translated from this open reading frame is cleaved to generate the individual viral proteins. In addition to the flaviviruses (genus *Flavivirus*), the family includes 2 other genera, the hepaciviruses (genus *Hepacivirus*, which includes hepatitis C virus and GB virus B) and pestiviruses (genus *Pestivirus*, which includes bovine viral diarrhea virus and classical swine fever virus). The 3 genera differ in the

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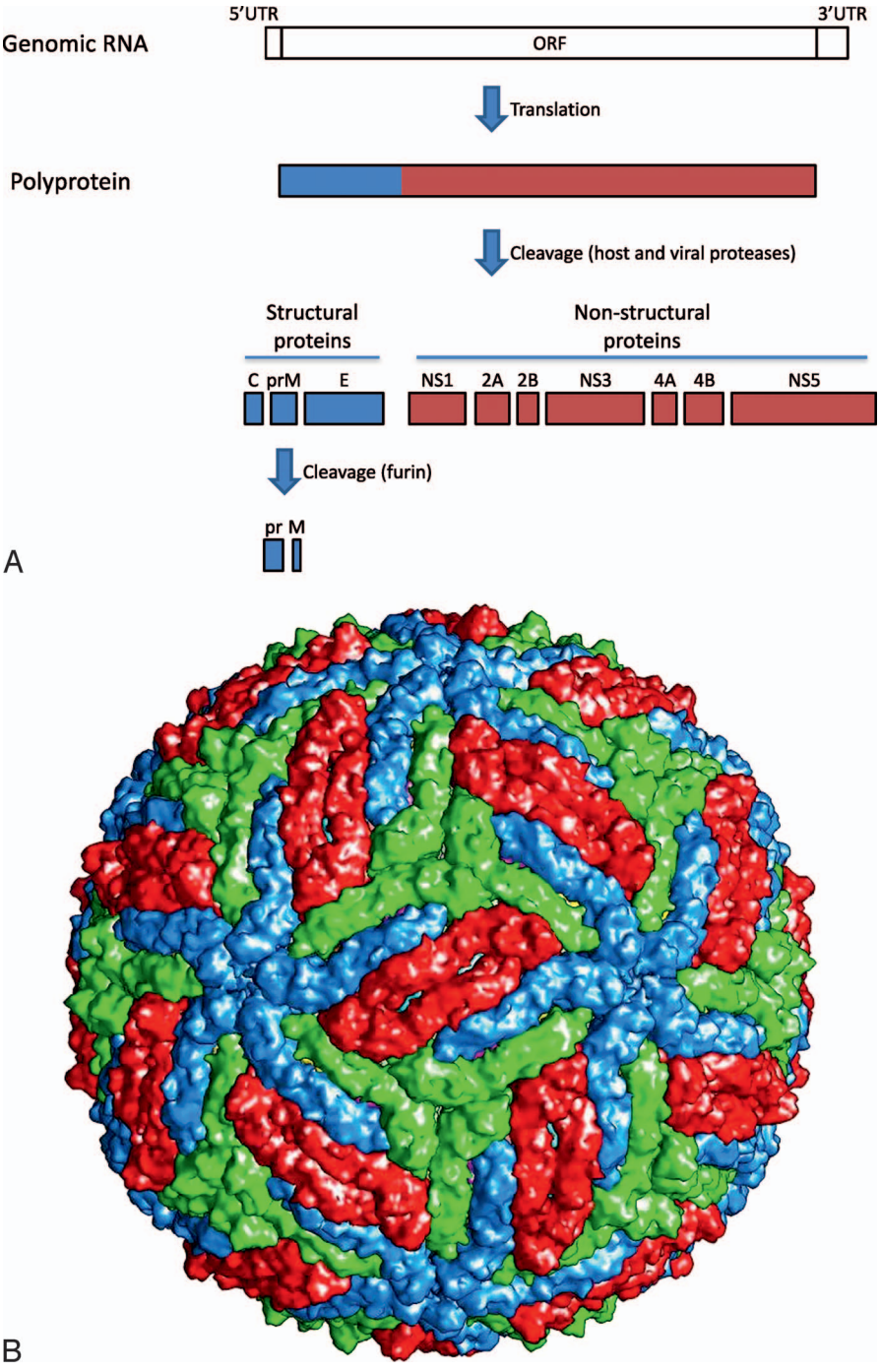

Figure 1. *Flavivirus molecular biology and structure. A, Schematic of the genome organization and protein expression strategy of flaviviruses. The genomic RNA is positive (message) sense, containing a single open reading frame (ORF) flanked by untranslated regions (UTRs). The ORF is translated to yield a single polyprotein that is processed by viral and host proteases to yield the 3 structural proteins (blue) and 7 nonstructural proteins (red); the N-terminus of NS4B is cleaved to yield a smaller protein referred to as 2K (not shown). Cleavage of premembrane (prM) occurs after virion assembly, during the process of virion maturation (see text). B, Structure of the mature flavivirus virion, showing the configuration of E glycoproteins on the surface. Individual molecules are colored based on their position at the 5-fold (blue), 3-fold (green), or 2-fold (red) axis of symmetry of the virion. The image shown was obtained from VIPERdb (http://viperdb.scripps.edu)[152] from coordinates from cryoelectron microscopy of the ZIKV virion.[153]*

complement of viral proteins (and their functions) and show little to no antigenic relatedness. Within a genus, the individual viruses encode homologous proteins with similar functions and variable degrees of antigenic homology.

More than 70 flaviviruses have been named to date; most have been associated with no or few human infections and were discovered through sampling of animals or arthropod vectors. The Table lists flaviviruses of medical importance along with key clinicopathologic characteristics.[13,14] The flaviviruses group phylogenetically in a pattern that follows their mode of transmission into mosquito-borne viruses, tick-borne viruses, and viruses with no known arthropod vector. Standards for defining individual members of the genus *Flavivirus* (and indeed, for other virus genera) are still evolving, because the genetic sequence database is expanding. Zika virus is a member of the mosquito-borne flaviviruses, as are several other viruses of medical importance, including yellow fever virus (YFV, after which the family is named), DENV, Japanese encephalitis virus (JEV), and West Nile virus (WNV). Zika virus is most closely related to the Spondweni virus,[15] and next most closely related to DENV.

Information on the genetic diversity among strains/isolates of ZIKV was extremely limited prior to 2014, but several phylogenetic analyses have since been performed in which strains have been clustered based on the nucleotide

sequences in more conserved regions of the genome (*NS5*, *NS3*, and *E* genes, which are described further below) or based on alignment with a reference viral genome.[5,16–21] Zika virus strains clustered into 2 major groups (clades)—an African lineage and an Asian lineage. The ZIKV strains responsible for the recent outbreak in the Americas form a distinct clade in the Asian lineage.[22]

Viruses in the genus *Flavivirus* have an RNA genome approximately 11 kb of RNA in length. The single open reading frame is flanked at both the 5′ and 3′ ends by untranslated regions that are important for translation and replication of the RNA genome through interactions with viral and host proteins (Figure 1, A).[23–26] The 5′ end of the viral RNA also has a type I cap structure that enhances translation of the RNA[27] and helps in evasion of the host immune response.[28–30]

The open reading frame encodes a polyprotein precursor, which is cotranslationally and/or posttranslationally cleaved by viral and host proteases. The amino-terminal one-third of the polyprotein yields the 3 flaviviral structural proteins, which are present in the virion particle- capsid (C), premembrane (prM), and envelope (E). The carboxy-terminal two-thirds of the polyprotein yields 7 (or 8) nonstructural proteins: NS1, NS2A, NS2B, NS3, NS4A, (2K), NS4B, and NS5, which are produced in infected cells and are involved in the viral life cycle but are not present in the virion particle.

The mature flaviviral virion is approximately 40 to 60 nm in diameter.[31,32] The major external feature of the mature virion is a tightly packed arrangement of 90 dimers of the E glycoprotein, which mediates binding to cells and fusion of viral and cellular membranes (Figure 1, B).[26] Although each dimer unit is structurally identical, their organization on the virion surface creates 3 distinct quaternary conformations with adjacent dimers at the 3-fold, 2-fold, and 5-fold axes of symmetry. The carboxy-terminal portion of each E monomer anchors the protein in the lipid envelope. A small fragment of the prM protein, in equal quantity to the E monomer, also remains embedded in the lipid membrane of the virion, the product of cleavage during virion maturation (see below). This fragment, termed M, is inaccessible to antibody binding. Inside the lipid membrane is the viral nucleocapsid, made up of the C protein and viral RNA. The C protein forms stable homodimers, which contain 4 alpha helices that interact with viral RNA and the viral membrane.[33,34] Dimerization and the N-terminal region of C are important for viral nucleocapsid and particle formation.[35,36] The nucleocapsid is relatively unstructured compared with the glycoprotein shell.[31,32,37]

The flavivirus nonstructural proteins perform multiple functions essential for viral replication, including processing the viral polyprotein, replicating the viral RNA, and inhibiting host immune responses. The NS5 protein is the largest viral protein; a larger C-terminal domain serves as the RNA-dependent RNA polymerase (replicase), whereas a smaller N-terminal domain functions as a methyltransferase that synthesizes the 5′ cap on new genomic RNA strands.[26] The NS3 protein is the next largest of the viral proteins. The N-terminal domain of NS3 is a serine protease, although its function requires interaction with the NS2B protein as a cofactor,[38–42] whereas the C-terminal domain of NS3 serves as both a helicase, which unwinds double-stranded RNA formed during viral genome replication, and a 5′-RNA triphosphatase, which is involved in formation of the 5′-RNA cap.[43–50] NS1 is a glycosylated protein that exists as multiple forms: a membrane-bound intracellular dimer involved in the viral replication complex, a GPI-linked form at the plasma membrane, or a hexameric form secreted from the infected cell.[51–53] The 4 small nonstructural proteins NS2A, NS2B, NS4A, and NS4B are associated with intracellular membranes, where they are involved in inducing membrane alterations, assembling the viral replication complex, and evasion of innate immune pathways.[54–67]

Recent studies of ZIKV have largely confirmed similarities with other flaviviruses in structure, genomic organization, and functions of the viral proteins. Some elements in the untranslated regions of the genomic RNA show less conservation in ZIKV compared with DENV2,[68] and some ZIKV strains show polymorphism (additions as well as deletions) in the length of the viral polyprotein[17,18]; the significance of these differences is not known.

THE FLAVIVIRAL LIFE CYCLE

Flavivirus infection is initiated when the viral RNA is introduced into the cytoplasm of the target cell after fusion of the virion envelope with endosomal membranes. Cellular mechanisms translate the viral structural and nonstructural proteins from the viral RNA, and the RNA is replicated by the viral replicase in conjunction with cellular factors. The newly synthesized viral RNAs are then packaged with viral structural proteins into a noninfectious immature particle, which leaves the cell via a cellular secretory pathway; during this process, virion maturation occurs. In the process of replication, the virus induces changes to both the subcellular structure and cellular metabolic pathways to promote its own replication and subvert host innate immune responses.

Entry

Flaviviruses bind to receptors at the cell surface and enter host cells by receptor-mediated endocytosis in clathrin-coated pits.[69,70] The principal receptors are not clearly defined for most flaviviruses, and several viruses appear to be able to use multiple different receptors.[71] Zika virus has been shown to be capable of infecting cells using the lectin DC-SIGN, which can also mediate infection by other flaviviruses, such as DENV.[72] Additional receptors reported for ZIKV include T-cell immunoglobulin and mucin domain (TIM1) TYRO3, and AXL.[72]

During endocytosis of the virion, acidification of the endosome triggers dissociation of the E protein dimers, exposing a hydrophobic peptide (fusion loop) and rearrangement of the E monomers into trimers. This fusion-active form of the E protein inserts into the endosomal membrane, inducing the fusion of the viral lipid envelope with the vesicle membrane and release of the viral RNA into the cytoplasm.

Because the role of receptor binding in flavivirus infections is to trigger endocytosis, a peculiar feature of flavivirus infections, at least in vitro, has been the ability of antibodies to increase viral uptake, a phenomenon referred to as antibody-dependent enhancement of infection.[73,74] Antibodies can block infection by inhibiting attachment, E protein rearrangement, or fusion, but this requires a critical threshold of antibody molecules bound to critical epitopes on the virion surface. Under conditions where the concentration of antibodies is low, antibody avidity is low, and/or antibodies target only nonneutralizing epitopes, antibody-bound virions remain infectious, and their attachment to

Flaviviruses Pathogenic to Humans			
Virus	Year of Isolation	Location	Source
Alkhurma	1995	Saudi Arabia	Human
Apoi	1954	Japan	Pooled rodents
Bagaza	1966	Central African Republic	*Culex* spp
Banzi	1956	South Africa	Human
Bussuquara	1956	Brazil	*Alouatta belzebul*
Dakar bat	1962	Senegal	Pooled bats
Dengue 1	1944	Hawaii	Human
Dengue 2	1944	New Guinea	Human
Dengue 3	1957	Philippines	Human
Dengue 4	1957	Philippines	Human
Ilheus	1944	Brazil	Pooled *Aedes* and *Psorophora*
Japanese encephalitis	1935	Japan	Human
Koutango	1969	Senegal	*Tatera kempi*
Kunjin	1960	Australia	*Culex annulirostris*
Kyasanur Forest disease	1957	India	*Presbytis entellus*
Langat	1956	Malaysia	*Ixodes granulatus*
Louping ill	1929	Scotland	*Ovis aries*
Modoc	1958	USA	*Peromyscus maniculatus*
Murray Valley encephalitis	1951	Australia	Human
Ntaya	1943	Uganda	Mosquitoes
Omsk hemorrhagic fever	1947	USSR	Human
Powassan	1958	Canada	Human
Rio Bravo	1954	USA	*Tadarida braziliensis mexicana*
Rocio	1975	Brazil	Human
St Louis encephalitis	1933	USA	Human
Sepik	1966	New Guinea	*Mansonia septempunctata*
Spondweni	1955	South Africa	*Mansonia uniformis*
Tick-borne encephalitis	1937	USSR	Human
Usutu	1959	South Africa	*Culex neavei*
Wesselsbron	1955	South Africa	*Ovis* spp
West Nile	1937	Uganda	Human
Yellow fever	1927	Ghana	Human
Zika	1947	Uganda	*Macaca mulatta*

Abbreviation: HF, hemorrhagic fever.

cells expressing immunoglobulin receptors (FcRs) may actually be enhanced; furthermore, signals via the FcR may render cells more susceptible to infection and enhance viral replication.[75] Antibody-dependent enhancement of infection is most readily observed in vitro under conditions where viral infection is inefficient and when using antibodies induced by closely related flaviviruses. It has been demonstrated most for DENV, where it has been thought to play a role in severe disease during secondary infection, but has also been demonstrated in vitro for other flaviviruses, such as YFV and WNV.[76,77]

Zika virus and DENV share the same mosquito vector, *Aedes aegypti*, and therefore circulate in the same geographic regions. Antibody-dependent enhancement of infection of ZIKV infection by cross-reactive antibodies from a previous DENV infection has been proposed to play a role of pathogenesis of ZIKV infection.[78] Sera from DENV-immune individuals and DENV-specific monoclonal antibodies have been shown to cross-react with ZIKV and enhance ZIKV infection in vitro via FcRs,[79] but the in vivo relevance of these findings is not known. In addition to being expressed on a wide range of immune effector cells, FcRs have been shown to be expressed by cell types within the central nervous system and the placenta.[80–88] Viruses such as HIV and cytomegalovirus can use maternal immunoglobulin (Ig) G to transcytose across the placenta to the fetus using FcRn.[89–92] These observations increase the concern that antibody-dependent enhancement of infection may be relevant to the risk of congenital ZIKV infection.

Replication

Flavivirus replication begins with recognition of the viral genome by the host protein synthesis machinery and translation of the RNA to yield the viral polyprotein. This process occurs at the endoplasmic reticulum (ER) membrane. Signal sequences at the N-terminus of the prM, E, and NS1 protein segments direct these newly synthesized portions of the polyprotein into the lumen of the ER, whereas the other viral proteins remain on the cytoplasmic side of the ER membrane. The polyprotein is cleaved by a combination of viral and host proteases; most cleavages between nonstructural proteins are performed by the NS3 protease domain in association with NS2B, whereas the signal sequence cleavages are performed by a host signalase. The viral nonstructural proteins, along with double-stranded RNA, locate to membranous vesicles that develop in the infected cell (Figure 2), suggesting that these vesicles are the sites of RNA replication.[57,93] Components of the NS2B3 viral protease complex localize specifically to convoluted membranes and paracrystalline arrays, which derive from rough ER, whereas viral double-stranded RNA, the replicative intermediate formed during synthesis of the complementary RNA strand, and NS5 localize primarily to vesicle packets, which are derived from *trans*-Golgi network membranes.[56,57,94–97]

The 5′ and 3′ untranslated regions of the viral genomic RNA contain secondary and tertiary RNA structures that interact to circularize the viral RNA genome and play an essential role in RNA replication.[24,98,99] The viral replication

Extended			
Geographic Distribution	**Principal Vector Species**	**Principal Host Species**	**Human Disease[a]**
Middle East	Ticks?	Livestock (cattle, camels, goats)	Fever, HF, encephalitis
Japan	?	Rodents?	Encephalitis
Africa	*Culex* spp	Unknown	Fever
Africa	*Culex* spp	Unknown	Fever
Brazil, Colombia, Panama	*Culex* spp?	Unknown	Fever
Africa	?	Bats?	Fever
Essentially worldwide	*Aedes aegypti*	Humans	Fever, rash, HF
Essentially worldwide	*A aegypti*	Humans	Fever, rash, HF
Essentially worldwide	*A aegypti*	Humans	Fever, rash, HF
Essentially worldwide	*A aegypti*	Humans	Fever, rash, HF
South and Central America	Mosquitoes	Birds	Fever
Asia	*Culex tritaeniorhynchus*	Birds	Encephalitis
Senegal, Central African Republic	Unknown	Rodents?	Fever, rash
Australia, Asia	*Culex* spp	Birds	Fever, rash
India	*Haemaphysalis spinigera*	Monkeys	HF
Malaysia, Thailand, Siberia	*I granulatus*	Unknown	Fever
UK	*Ixodes* spp	Sheep	Encephalitis
USA	?	*P maniculatus*	Encephalitis?
Australia, New Guinea	*C annulirostris*	Birds	Encephalitis
Africa	Mosquitoes	?	Fever
Western Siberia	*Dermacentor pictus*	Muskrats	HF
Canada, USA, USSR	*Ixodes* spp	Small mammals	Encephalitis
USA, Mexico	?	*T braziliensis mexicana*	Fever
Brazil	Mosquitoes	Birds	Encephalitis
The Americas	*Culex* spp	Birds	Encephalitis
New Guinea	Mosquitoes	?	Fever
Africa	*Aedes circumluteolus*	Unknown	Fever
Europe, Asia	*Ixodes* spp	Rodents	Encephalitis
Africa	Mosquitoes	Birds?	Fever, rash
Africa, Asia	*Aedes* spp	Unknown	Fever
Worldwide	Mosquitoes	Birds	Fever, rash
Africa, South America	*A aegypti*	Monkeys	Pantropic
Africa, Asia	*Aedes* spp	Monkeys?	Fever, rash

complex carries out RNA synthesis, RNA capping, and 5′ RNA methylation.[100–102] Multiple host proteins are also involved in this process.[103]

Although viral replication occurs in the cytoplasm, some viral proteins traffic to other subcellular locations, suggesting additional functions during infection. As noted above, some NS1 protein is secreted from the infected cell as a hexamer; for DENV, this has been proposed to contribute to activation of the complement cascade and other immune response pathways and to vascular leakage. Also for DENV, both C and NS5 proteins have been shown to traffic to the nucleus; the significance of this finding is not fully understood, but it has been proposed to influence gene expression.[104,105] Little is known about the trafficking of ZIKV proteins; however, one older study did demonstrate staining for ZIKV E protein in the nucleus of infected cells.[106]

Viral Particle Assembly and Maturation

Virion formation occurs in the cytoplasm of infected cells. In their initial configuration, referred to as immature virions, particles contain a lipid membrane with full-length prM and E proteins arranged in an icosahedral lattice on the virion surface. The prM and E proteins are released at the luminal side of the ER during the translation and processing of the viral polyprotein, where 3 molecules of each protein interact to form trimers of prM-E heterodimers.[107,108] The C protein, which contains an RNA-interacting domain, anchors to the cytoplasmic side of the ER membrane by a C-terminal transmembrane domain that also serves as the signal sequence for prM translocation into the ER lumen.[109] Once the nucleocapsid begins to form, prM interacts with C, which leads to budding of the immature virion into the lumen of the ER.[110]

The virion remains in this immature conformation in the infected cell until it moves through the acidic compartment of the *trans*-Golgi network. The decrease in pH induces cleavage of prM by the host protease furin, which leads to rearrangement of the virion surface into the herringbone-like lattice of E protein dimers of the mature virion, although the remaining portion of prM (pr peptide) still remains associated with the virion.[111,112] The particle is then exported from the cell using the host secretory machinery, and the increase in pH to physiologic levels causes the pr peptide to dissociate from the now mature virion.

Cryoelectron microscopy studies indicate that the mature ZIKV virion is very similar in structure to the mature virions of DENV and WNV.[32,113,114] The major difference between DENV and ZIKV lies in the glycosylation of the E protein; DENV E is glycosylated at 2 sites (Asn57 and Asn153), whereas ZIKV has a single glycosylation site at Asn154.[113] Previous work on WNV showed that Asn154 glycosylation enhanced viral neuroinvasiveness.[115] It remains to be determined whether ZIKV neurotropism is similarly associated with glycosylation of Asn154.

Induction and Inhibition of Innate Immune Pathways

Flaviviruses are detected in cells by innate immune pathways called pattern recognition receptors that recognize pattern-associated molecular patterns. The activation of pattern recognition receptors induces signal cascades that

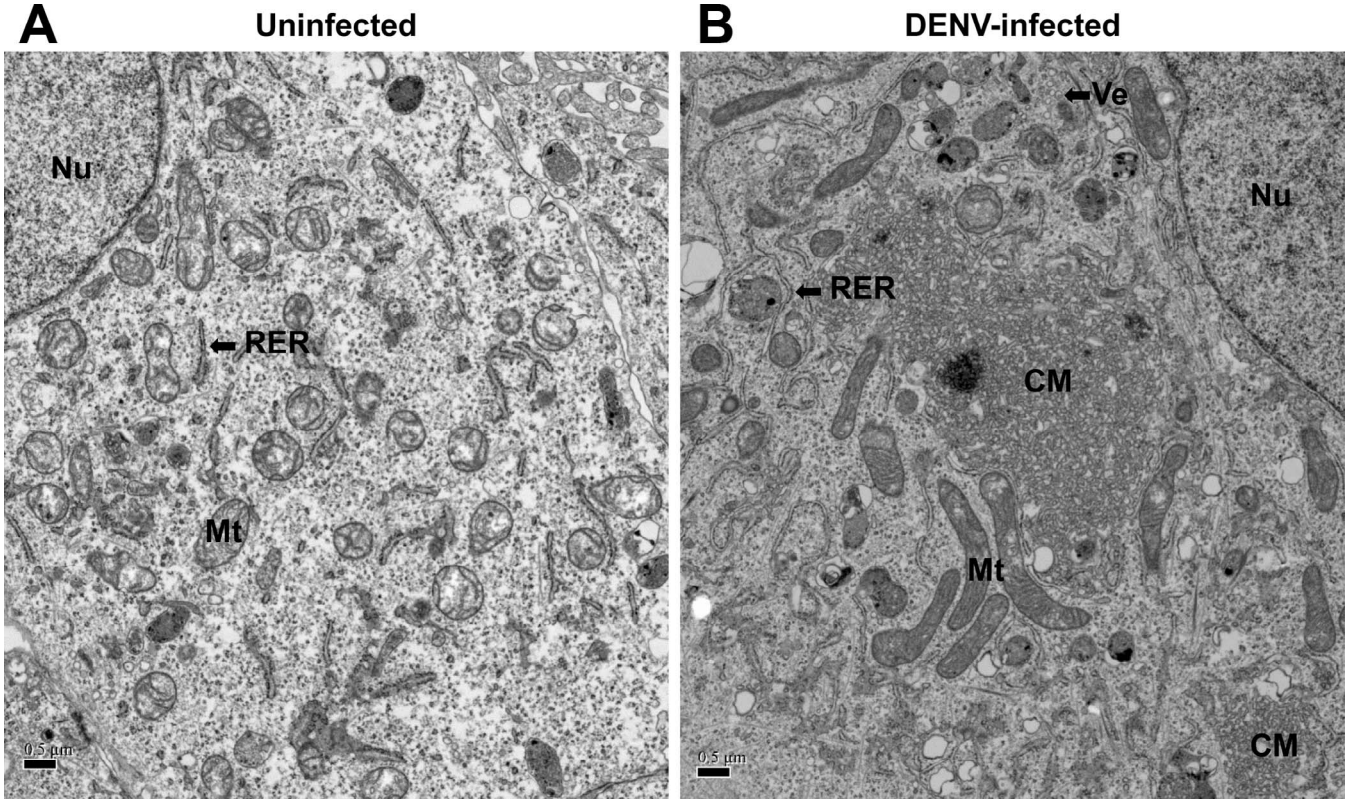

Figure 2. *Cellular changes during flavivirus infection. Ultrathin section transmission electron microscope images of uninfected Huh7 human hepatocytic cells (A) and Huh7 cells infected in vitro with dengue virus (DENV; B) (original magnification ×7900). Abbreviations: CM, convoluted membranes; Mt, mitochondria; Nu, nucleus; RER, rough endoplasmic reticulum; Ve, vesicles.*

lead to the production of an antiviral response, mainly through expression of type I and type III interferons (IFNs). Flaviviruses, such as DENV, activate pattern recognition receptors, including RIG-I/MDA5 and TLR3,[116] and some evidence suggests that the DNA sensor cGAS may also play a role in defense against DENV.[117] Zika virus infection of human fibroblasts increased the expression of TLR3, RIG-I, and MDA5 RNA.[72] The downstream expression of type I IFN–stimulated genes was also increased, indicating activation of the pattern recognition receptors. The IFN-stimulated gene IFITM3, a small membrane-associated IFN-inducible transmembrane protein 3, was shown to inhibit flaviviral replication.[118] Recently, IFITM3 and IFITM1 were also shown to inhibit the replication of ZIKV.[119] Another report showed that type III IFN, IFNλ1, constitutively secreted by placental trophoblasts, protected cells from ZIKV infection.[120] Antiviral activity was not detected when ZIKV-infected cells were treated with IFNλ1, however, suggesting that ZIKV may antagonize IFNλ1 signaling, as was seen for type I IFNs during DENV infection.[121]

CLINICOPATHOLOGIC CORRELATIONS OF VIROLOGY

The principal clinical syndromes associated with flavivirus infections are nonspecific febrile illnesses with or without rash, viral hemorrhagic fevers, and neurologic diseases (Table). Murray Valley encephalitis, JEV, St Louis encephalitis, WNV, and tick-borne encephalitis are noted for their tendency to cause central nervous system infections, particularly encephalitis.[122,123] Dengue fever virus has been associated with neurologic involvement in some patients, although much less often than febrile illness or hemorrhagic fever.[124–128] No single viral protein has been shown to determine neurovirulence across flaviviruses; within individual flavivirus species, particular amino acids in E, NS1, NS3, and NS5 proteins may be involved.[129–136]

Tropism

Human skin explants have been shown to be permissive to ZIKV infection and may constitute an important initial site of infection.[72] Zika virus RNA is detected in plasma and urine samples from patients infected with ZIKV, suggesting involvement of other organs once infection is established.[137] Of greatest concern is the detection of ZIKV in amniotic fluid and fetal brain tissue, indicating that ZIKV crosses not only the placenta but also the fetal blood-brain barrier.[138,139] Hofbauer cells and histiocytes stained positive for ZIKV antigen in placentas, indicating that the virus is able to infect the placenta in addition to crossing it. Zika virus proteins were found in glial cells and in endothelial cells within the brain tissue of newborns or miscarriages with microcephaly or ZIKV infection during pregnancy.[140] Zika virus RNA is detected in the semen and urine of patients, suggesting infection of cells of the genitourinary tract, which is thought to explain the finding of sexual transmission of ZIKV.[141–143]

In vitro studies have found many cells that can be infected with ZIKV. Human dermal fibroblasts, epidermal keratinocytes, and immature dendritic cells were infected by ZIKV.[72] Zika virus has also been shown to infect primary human Hofbauer cells (placental macrophages) and cytotrophoblasts, whereas syncytiotrophoblasts appear to be resistant to infection.[120,144] Zika virus infects human neural progenitor cells derived from induced pluripotent stem cells.[145] The

effect of ZIKV infection on other cells of the central nervous system remains unclear.

Diagnosis

Diagnostic testing for flaviviruses uses either direct detection of the virus (or its products) or detection of antibody responses to viral infection. Virus can be detected from blood, body fluids, or tissues, depending on the viral tropism and stage of infection. For ZIKV, urine appears to have a higher yield for viral detection than blood.[146] Cell culture detects infectious virus particles, but it is time-consuming and not widely available. Reverse transcriptase–polymerase chain reaction methods detect both intact viral particles (both infectious and noninfectious) as well as replicating viral RNA inside cells. Primer design and reaction conditions influence both the sensitivity and specificity of the assay; the high error rate of RNA replication can introduce mutations that interfere with polymerase chain reaction amplification, whereas partial sequence homology with other flaviviruses can lead to "false-positive" amplification. Soluble NS1 protein, which is secreted from infected cells, can be detected in blood and some other specimens using enzyme-linked immunosorbent assays or lateral flow immunoassays, and this has been exploited for the diagnosis of DENV infection.[147] The specificity of capture and detection antibodies is a critical factor in determining if other flaviviruses can give "false-positive" reactivity.

Plaque Reduction Neutralization Test (PRNT) and IgM enzyme-linked immunosorbent assay are the serologic assays currently available for ZIKV.[148] Both assays use intact virions and mainly detect antibodies directed at the E protein. For DENV, IgM antibodies are typically detectable from the fifth day of illness onward, and they remain positive for several months; a similar pattern can be expected for ZIKV infection. The assay is fast, low cost, and provides a high throughput. However, the interpretation of the assay is confounded by antibody cross-reactivity with other flaviviruses, particularly DENV. The IgM response can also be blunted during a secondary flavivirus infection, reducing the sensitivity of this assay. The PRNT has a greater ability to discriminate between virus-specific and cross-reactive antibodies induced by infection with related flaviviruses, because strongly neutralizing antibodies are often directed at more specific epitopes. For further evaluation of patients with a positive IgM enzyme-linked immunosorbent assay result, the finding of ZIKV-specific PRNT titers that are 4-fold or higher than DENV PRNT titers is supportive of recent ZIKV infection.[149] However, considerable cross-reactivity is still observed in the PRNT, especially in patients with prior exposure to another flavivirus, and this assay has not proven to provide a definitive diagnosis.

Vaccines

Vaccines for human use are currently available for YFV, JEV, tick-borne encephalitis virus, and DENV[150,151]; the tick-borne encephalitis virus and DENV vaccines are not licensed in the United States. All are whole-virus vaccines, either live or inactivated. The live attenuated YFV vaccine strain was isolated after serial passage in embryonated chicken eggs, whereas the 4 live attenuated DENV vaccine strains are chimeric flaviviruses developed by replacing the prM and E gene segments in the YFV vaccine virus with homologous sequences of each DENV type using recombinant DNA techniques. The tick-borne encephalitis virus and JEV vaccines contain chemically inactivated viruses, but a live attenuated JEV vaccine is also available outside the United States. The mechanism of protection from these flavivirus vaccines is not fully understood. Neutralizing antibodies serve as a correlate of protection for YFV and JEV vaccines. For DENV, although higher neutralizing antibody titers are associated with resistance to infection and/or disease, the relationship is more complex, probably related to cross-reactivity between DENV types. Based on analogy to other flaviviruses, the induction of neutralizing antibodies directed at the ZIKV E glycoprotein should be the main objective for efforts to develop an effective vaccine. Discriminating protective responses to ZIKV from cross-reactive antibody responses to other flaviviruses, especially DENV, will be a major challenge and is a focus for the development of better assays of ZIKV-specific antibodies.

CONCLUSIONS

This review has attempted to provide a basic introduction to the composition, structure, and biology of ZIKV, drawing on observations from the related flaviviruses and highlighting novel findings on ZIKV where available. Given the intense scientific effort directed at understanding the unique characteristics of ZIKV, including sexual transmission and congenital infection, the knowledge base is rapidly expanding and it can be expected that some perspectives will further evolve. Although advances in basic virology studies of ZIKV will undoubtedly generate great interest, contributions from clinical and anatomic pathologists will continue to be essential to place these studies in the proper perspective.

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