Chapter 6
Chikungunya Virus and Zika Virus Expansion: An Imitation of Dengue Virus

6.1 Introduction

Chikungunya virus [CHIKV] and dengue viruses [DENV] have many similarities in clinical manifestations and epidemiology, as well as means of transmission by *Aedes* species of mosquitoes. Moreover, the emergence and pattern of spread of dengue virus disease [DVD] from tropical and subtropical regions of the world to a global dispersal more than 50 years ago is being repeated by CHIKV in the last few years. Although both conditions are largely self-limited, systemic viral infections transmitted primarily between humans and mosquitoes, they evolve from Africa or Asia as zoonotic infections between nonhuman primates and other small animals with humans as secondary hosts. Presently, endemic and epidemic outbreaks of dengue viruses and CHIKV are through human-to-human transmission by mosquitoes, rather than enzootic or epizootic means. The spread of these viruses globally is directly related to the dispersal and adaptation of the vectors, *Aedes aegypti* and more recently *Aedes albopictus*. The primary vector, the urban adapted *Ae. aegypti mosquito,* is widely distributed in tropical and subtropical regions of the world. It is believed that this vector emerged from Africa during the slave trade in the fifteenth to the nineteenth centuries to the Americas and Caribbean, spread through Asia during commercial trading in the eighteenth and nineteenth centuries, and spread more globally in the past 50–60 years with increased international travel and trade [1]. *Ae. aegypti* was initially introduced in Europe during the seventeenth to nineteenth centuries where it existed in southern Europe until its disappearance during the twentieth century and has since returned on Madeira and the Black Sea Coast [2]. *Ae. aegypti* eggs may have been transmitted to the Americas by water containers on slave ships, and now it is believed that larvae and eggs of the Asian tiger mosquito, *Ae. albopictus,* were imported into the western hemisphere from Japan through trade of used car tires, where the mosquitoes lay its drought-resistant eggs in collected rainwater [3]. Although the burden global of dengue is estimated between 50 and 100 million cases a year affecting about 100

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countries with over 2.5 billion people at risk [4], there is no similar estimate of the global burden or annual incidence of CHIKV disease as outbreaks are more variable, and small sporadic outbreaks are often attributed to dengue. Zika virus, also transmitted by *Aedes* species of mosquitoes, was recognized to cause mild sporadic disease in Africa and Asia for decades until its emergence in the southwestern Pacific Ocean in 200 and sudden explosive outbreak throughout the Americas and Caribbean in 2015–2016.

6.2 Historical Aspects

6.2.1 CHIKV

CHIKV was first isolated from febrile patients during an outbreak in the southern province of Tanzania [Makonde Plateau] in 1952–1953 [5]. The name chikungunya was derived from the Swahili or Makonde word meaning "to become contorted" or "that which bend up," due to the severe muscle and joint pains that can continue for years and may be so severe that some patients adopt a bent or stooping position [5]. *Ae. aegypti* is the primary mosquito vector in Africa and other tropical or subtropical countries, but multiple other *Aedes* species have been implicated in Africa, where CHIKV is maintained in a sylvatic cycle among mosquitoes, wild primates, and other small mammals [rodents, bats, and squirrels] and birds [6]. However, urban transmission from virus circulating in eastern Africa in nonhuman primates to humans had occurred on multiple occasions [7]. Outbreaks of CHIKV infection across Africa were associated with heavy rainfall in rural forested areas with increased mosquito population and spillover from the enzootic forest cycle to epizootic savannah cycle, with exposure to nonimmune populations. During epidemics the CHIKV can circulate between human populations without the presence of animal reservoirs [8]. Subsequent emergence and spread of the virus beyond Africa are estimated to have occurred in the eighteenth century, with carriage of infected mosquitoes aboard sailing ships along with barrels of stored water and with humans to maintain the propagation of the mosquitoes and a local cycle [9]. CHIKV of African lineage is believed to have been introduced in Asia in the nineteenth century or sometimes between 1879 and 1956, after adaptation into urban cycle [10].

Outbreaks of CHIKV in Asia were first recognized in 1954 from the Philippines with subsequent outbreaks in 1956 and 1968 [8]. During the 1970s and early 1980s, the virus spread throughout southern and Southeast Asia, with frequent epidemics and sporadic activity in many Asian countries, but eventually the virus activity dwindled and ceased for many years with only localized outbreaks. After several decades of inactivity, CHIKV reemerged in 2000 as an urban epidemic in Kinshasa [Democratic Republic of the Congo] after 39 years of absence [11] and reappeared in Indonesia after 20 years of inactivity in 2001–2003 [12]. In 2004, an outbreak involving the ECSA [eastern, central, and southern African] lineage progenitor began in the coastal towns of Kenya and spread to several Indian Ocean Islands and

onto India where it caused massive outbreaks of several million people [13]. Infected air travelers from the Indian Ocean basin outbreaks retuned to Europe, Australia, the Americas and other parts of Asia, and secondary local transmission resulted in Italy, urban France, South and Southeast Asia [10]. The extent of the explosive outbreaks in the Indian Ocean basin has been attributed partly due to the ease of air travel which facilitated rapid spread, large dense urban populations previously naïve to CHIKV exposure and vulnerable to infections, dense populations of *Aedes* mosquitoes with introduction of *Ae. albopictus* mosquitoes into this region from native Asia islands in 1985, and adaptive mutations of the new Indian lineage CHIKV strains which resulted in enhanced transmission by the new mosquito vector [14–16]. *Ae. albopictus* mosquito species were not implicated as a major vector in prior Asian epidemics and older Asian lineage of CHIKV was not well adapted to this mosquito [17].

During the outbreaks of the Indian Ocean lineage [IOL] of CHIKV between 2006 and 2009, many infected travelers returned to the Americas without provoking local transmission, despite many imported cases [18]. However, in October–December 2013, a single case of an Asia-lineage CHIKV infection was introduced in the island of St. Martin and rapidly spread across the western hemisphere. It is estimated that more than 1.2 million cases of CHIKV infection occurred locally from 44 countries and territories throughout the Americas, except for Canada [19]. Local transmission was reported throughout almost all Caribbean islands, all countries in Central America, several South American countries, and parts of Mexico and the USA [20]. In 2014, a total of 2811 CHIKV cases were reported from US states with 12 locally transmitted in Florida; all other cases were from returning travelers from affected countries [21]. ArboNET [the national surveillance system for arthropod-borne disease] reported 4710 cases from US territories for 2014, mainly locally transmitted cases from Puerto Rico, US Virgin Islands, and American Samoa [21].

6.2.2 DENV

The site of origin of DENV is controversial as some consider an African origin and being the same as the principal vector, *Ae. aegypti*, while others contend an Asian origin [22]. Early Chinese literature described dengue-like illness during the Chin Dynasty [Common Era 265–420] and other subsequent dynasties, and seven centuries later, a similar illness appeared in the French West Indies and Panama in the seventeenth century [22]. Possible dengue pandemic occurred in the latter part of the eighteenth century with widespread geographic distribution from present-day Jakarta to Philadelphia. Benjamin Rush has been attributed to provide the first detailed clinical description of DENV infection, as well as the as the application of the term "breakbone fever," in an epidemic in Philadelphia in 1879 [22]. The discovery that dengue fever was due to a virus was from experiments in human volunteers at Fort McKinley in the Philippines in 1907 [23]. *Ae. aegypti* mosquito was

confirmed to be the primary vector by 1926 [24] and subsequently *Ae. albopictus* was incriminated as well in 1931 [25].

Records suggest that there were at least five dengue pandemics from Africa to India/Oceana and to the Americas from 1823–1916, each lasting 3–7 years and propagated by the slave trade and commerce with entry via seaports of coastal regions [22]. After the World War 1, the pattern of DENV infections changed in Southeast Asia, Indian subcontinent, and the Philippines from episodic epidemics to persistent endemic state, but the Caribbean and the Americas remained intermittently active. By the late 1920s, major epidemics occurred throughout the Gulf and Atlantic states, the Caribbean, South Africa, Egypt, and Greece, affecting millions of people. With eradication of *Ae. aegypti*, DENV was eliminated from the Mediterranean region by the mid-1940s [22]. By the 1960s and 1970s, there was dramatic increase in dengue activity in tropical and subtropical regions of the world, and by the 1990s there was a global distribution of all DENV serotypes to urban populations. Since the arrival of the twenty-first century, the annual incidence of dengue fever and intensity of epidemics have dramatically increased around the world. Several major outbreaks have occurred in this century in the Americas, Southeast Asia, and Asia, and all DENV serotypes have global hyperendemicity, and it is expected that recurrent outbreaks will continue possibly in cycles of 3–5 years, with annual mortality of 20,000 or more [22].

Although most dengue infections arise from human reservoirs, the ancestral DENVs are believed to have circulated in tropical forests to maintain the cycle among nonhuman primates and mosquitoes. The sylvatic DENVs are genetically and ecologically different from the urban viruses responsible for most endemic and epidemic outbreaks. Sylvatic cycles of DENV have been demonstrated in Asia and Africa between canopy-dwelling mosquitoes and tree-dwelling animals, such as monkeys, slow lorises, civets, and squirrels [26, 27].

6.2.3 Zika Virus

Zika virus [ZIKV] was first discovered in 1947 after a rhesus monkey developed a febrile illness, while caged as a sentinel animal on a tree platform in the Zika forest of Uganda, for research on jungle yellow fever [28]. ZIKV was subsequently isolated from *Aedes africanus* mosquitoes from the same forest in 1948 [29]. Although serological studies in the early 1950s indicated that humans could be infected with ZIKV, the virus was first isolated from humans in 1968 in Nigeria and from 1971 to 1975 [30, 31]. Subsequent studies from 1951 to 1981 found serological evidence of ZIKV infection throughout Africa [Uganda, Tanzania, Egypt, Central African Republic, Sierra Leone, and Gabon] and parts of Asia [India, Malaysia, the Philippines, Thailand, Vietnam, and Indonesia] [32]. ZIKV was isolated from *Ae. aegypti* mosquitoes in Malaysia in 1968–69 [33], and since then it has been considered the primary vector for the virus. Infection with ZIKV first emerged outside Africa and Asia in 2007, when it caused an outbreak in the southwestern Pacific Ocean Yap Island, of the Federated States of Micronesia [34]. Since then it has

spread to other Pacific islands, French Polynesia in 2013–2014, Caledonia, Cook Islands, Cape Verde, and then Easter Island [Chile], and subsequently to Brazil and Columbia [35]. Since 2015–early 2016, an explosive outbreak has occurred in the Americas and Caribbean with over 30 countries affected, and it is predicted that by the end of 2016, four million people could be affected [Amedeo Zika Virus, http://amedeo.com/medicine/zik.htm]. By February 1, 2016, the WHO declared ZIKV a global public health emergency because of the reported association of microcephaly in newborns of infected pregnant women in Brazil.

As of August 2016, there have been 29 locally acquired mosquito-borne cases of ZIKV infection in Florida and 2487 travel-associated cases in the USA, of which 22 were sexually transmitted and 7 Guillain-Barre syndrome-associated cases [CDC. Case counts in the US. Accessed August 24, 2016 at http://www.cdc.gov/ncezid]. The ZIKV progression globally is circling back to Asia, as there is now an outbreak in Singapore with over 200 cases and a few cases in Malaysia [CNN broadcast, September 5, 2016]. In the past year, 2015–2016, Puerto Rico had experienced a ZIKV outbreak with at least 28,219 confirmed cases [36]. Moreover, recently locally transmitted cases of ZIKV were reported in Brownsville, Texas by CDC on Dec 14, 2016 [https://www.cdc.gov/media/releases/2016/s1214-brownsville-texas-zikaguidance.html].

6.3 Virology

6.3.1 CHIKV

Phylogenetic studies indicate that there are three CHIKV genotypes: Asian, East/Central/South African, and West African [37]. It has been estimated that the African genotypes emerged between 100 and 840 years ago, while the Asian genotype between 50 and 310 years ago [38]. CHIKV belong to the genus *Alphavirus* of the *Togaviridae* family and is grouped together with the Semliki Forest antigenic complex alphaviruses such as Ross River virus and others, which are also mosquito-borne [38]. CHIKV and other alphaviruses have single-stranded, positive-sense RNA genome which encodes four nonstructural proteins and three main structural proteins, the capsid and two envelope glycoproteins [10]. Unlike DENV there is limited antigenic diversity between the three genotypes of CHIKV, and previous infection seems to provide lifelong immunity [8].

6.3.2 DENV

DENV belong to the genus *Flavivirus*, family *Flaviviridae,* with four distinct but antigenically related serotypes [DENV-1–DENV-4], and each serotype has closely related multiple genotypes [22]. A potential fifth serotype [DENV-5] was isolated from a patient in Borneo, but it is unclear if this strain can cause sustained

transmission between humans [39]. Infection with a particular serotype will usually produce lifelong immunity to that serotype and associated genotypes, but cross protection against other serotypes is generally weak and short-lived, lasting for 2–3 months [40]. Dengue virions are spherical, about 50 nm in diameter with a host-derived lipid bilayer containing a single-stranded, positive-sense RNA genome coding for three structural and seven nonstructural proteins [40]. Ancestral dengue virus arose 1000–2000 years ago among monkeys in either Africa or Asia, and it is estimated that DENV-1 and DENV-2 emerged more recently, within the past three centuries [41]. Current evidence supports the hypothesis that all four endemic DENVs evolved from sylvatic progenitors in the forest of Asia and only DENV-2 could have emerged from African jungles [22]. The emergence of endemic DENV with humans as the only reservoir may have occurred within the past 2000 years and theoretically should be controllable with an effective vaccine. However, sylvatic DENV still exist in the forests of Asia and Africa, and fitness studies of sylvatic DENV-2 compared with human-circulating endemic/epidemic strains suggest that little genetic adaptation was required for the initial emergence of endemic strains. Hence sylvatic reservoirs in Asia and Africa will remain as a source of reemergence [22].

6.3.3 ZIKV

ZIKV is a flavivirus related to DENV, yellow fever, West Nile, and the Japanese encephalitis viruses, and it is a positive-sense single-stranded RNA virus with 10,794 nucleotides encoding 3419 amino acids [31]. There is only one strain recognized to date with three to five clades based on site and place of isolation, i.e., Zika Uganda 47, Senegal 84 [a, b, c], and Yap 2007. Based on phylogenetic analysis of ZIKV sequences, there are only two main virus lineages [African and Asian], and the strain responsible for the Yap Island and Pacific Ocean epidemics and the outbreak in the Americas likely originated in Southeast Asia [42, 43]. It has been estimated that ZIKV emerged in East Africa around 1920, and in the late 1940s, the seropositivity was 6.1% of the population in Uganda, and by the late 1960s, Kenya demonstrated seropositivity of 52% [44]. ZIKV has been isolated from several species of *Aedes* mosquitoes and wild monkeys, which are considered the natural hosts. In Africa ZIKV still maintains a sylvatic transmission cycle between nonhuman primates by species of *Aedes* mosquitoes, but in Asia this has not been demonstrated but possibly exists. It was demonstrated in the laboratory that infected *Ae. aegypti* mosquitoes could transmit ZIKV to mice and monkeys and that the extrinsic incubation period in mosquitoes was about 10 days [45]. The virus can be isolated from monkeys 9 days after inoculation and in humans the first day of illness and can be detected up to 11 days after onset [31, 33]. *Ae. aegypti* mosquitoes are the main vectors of ZIKV, but it is likely the virus can also be transmitted by *Ae. albopictus*. Most of the ZIKV transmission is from human to mosquito-human spread of the virus, but transmission of infections has been documented through intrauterine infection of the developing fetus, sexual transmission, potential blood transfusion, and laboratory exposure [46–49]. Sexual transmission from male returning travelers

who acquired infection in South America has been reported, and this may occur before, during, and after development of symptoms [50, 51]. High viral RNA or replicative viral particles have been detected in sperm up to 62 days after onset of symptoms [52, 53]. Preliminary data [one case] indicate that ZIKV can be transmitted by body fluids such as tears or saliva, when the viral load is extremely high [54]. MMWR Morb. Mortal. Wkly Rep. 2016 Sep. 13; e-pub [http://www.cdc.gov/mmwr/volumes/65/wr/mm653e4.htm].

6.4 Vectors of CHIKV, ZIKV, and DENV

Ae. aegypti, the primary mosquito vector for DENV, ZIKV, and CHIKV, is distributed worldwide in tropical and subtropical regions between 35°N and 35°S [latitude], with the lowest winter temperature of 10 °C in regions where the species can survive overwintering [55]. Expansion of *Ae. aegypti* population globally appears to have occurred after the end of eradication programs in the 1970s with return of the pre-eradication levels by 1995 [56]. The mosquito was previously widespread in southern Europe where it caused local outbreaks of yellow fever in the nineteenth century. It is well adapted to the urban environment and breed in small puddles of water and water collected in cans, empty containers, or old car tires in yards or homes. The female mosquitoes are the vectors of these viruses, and they feed on human hosts from early morning to just before sunset. After acquiring the virus from an infected host, there is an incubation period of 4–10 days and the mosquitoes remain infectious for their life-span [56]. Although the mosquitoes have limited range of flight, about 500 m, they are capable of feeding on multiple persons in a short period of time, and the movement of the infected individuals throughout and between the communities may help drive epidemics [56]. *Ae. albopictus,* another urban or domestic mosquito, is considered a secondary vector for DENV and CHIKV and has been implicated in previous epidemics of both viruses. *Ae. aegypti* had been eradicated by DDT in some regions and *Ae. albopictus* emerged to cause outbreaks of DENV and even larger epidemics of CHIKV in the islands of the Indian Ocean between 2006 and 2006 [57]. It appears that CHIKV had undergone adaptive mutations to *Ae. albopictus* to allow for greater transmissibility with more efficient crossing of the mosquito gut membrane barrier [15]. *Ae. albopictus* tolerates a wider range of temperature variation and environment than *Ae. aegypti*, from tropical to temperate climate, due to diapause or mechanism for tolerating harsh winters [57]. Although *Ae. aegypti* is endemic in some southern US states [Florida, Texas, and California], *Ae. albopictus* has spread to 36 states with its northernmost boundary in the northeastern USA including parts of New Jersey, southern New York, and Pennsylvania [58]. This mosquito is endemic in southern Europe where it has caused local outbreaks of CHIKV infection and colonized almost all Mediterranean countries [59]; besides its widespread distribution includes throughout the Americas [excluding Canada], Europe, Asia, Africa, Australia, and the Pacific [41]. In dengue endemic areas, *Ae. aegypti* is considered the major vector for transmission, and *Ae. albopictus* is considered less efficient at transmitting the

virus; however, some studies suggest that the latter may be more effective at dengue transmission because of the longer life-span of this species [60]. It has also been suggested that *Ae. albopictus* is less adapted to the urban environment than *Ae. aegypti* and may be involved in maintaining the sylvatic and rural transmission in endemic areas [61]. In a recent unique study, the direct susceptibility of these two species of mosquito to dengue infection after feeding on viremic humans was compared, *Ae. albopictus* was significantly less likely than *Ae. aegypti* to develop an infectious phenotype 14 days after direct feeding for DENV-2 and DENV-4 but not for serotypes 1 and 3 [62].

Various *Aedes* species had been found to be infected with ZIKV and are potential vectors of the virus [63], although transmission is believed to be mainly by *Ae. aegypti.* However, *Ae. albopictus* [which is capable of transmitting over 20 arboviruses] has been shown in the laboratory to be capable of transmitting ZIKV [64]. Furthermore, there is evidence that *Ae. albopictus* carried ZIKV in the wild and likely transmitted the virus to humans in Africa [65].

6.5 Pathogenesis of Disease

6.5.1 CHIKV Disease Pathogenesis

Currently the mechanisms involved in the pathogenesis of CHIKV disease are incompletely understood. The virus is capable of infecting and replicating in human endothelial cells, fibroblasts, and macrophages but cannot replicate in lymphocytes, monocytes, and dendritic cells, and replication was associated with cytopathic effect and induction of apoptosis in infected cells [66]. In subjects with severe CHIKV infection, increased proinflammatory cytokines, interleukin [IL]-1 and IL-6, are associated with severity of disease [67], and dysregulation of the inflammatory response may be inducing the disease manifestations. Moreover, the virus can infect and replicate in human muscle cells with inflammatory reaction which can explain the symptoms of CHIKV-induced myositis [68]. Studies in mice and nonhuman primates showed that the virus replicates in high concentration in joint tissues with associated influx of inflammatory cells, mainly monocytes, macrophages, and natural killer cells, and foot swelling in the mouse was reduced with macrophage depletion [69]. Furthermore, in the primate model during the acute phase of CHIKV infection, the virus disseminates to the lymphoid tissue, liver, central nervous system, joints, and muscle, and chronically [44 days postinfection], there is low-grade persistence of the virus in splenic macrophages and endothelial cells of the liver sinusoids [70]. In the mouse model, type 1 interferons [IFNs] are upregulated and are important in controlling the infection, and type 1 IFN receptor-deficient mice are more susceptible to severe disease [71]. Neuroinvasion and replication in the brains of infected mice are also seen with pathological changes in the brain parenchyma and meningeal inflammation and associated neurological signs [72].

6.5.1.1 Immune Response to CHIKV

Type-1 IFNs, as part of the innate immunity, are central to the control of viral infections including CHIKV [73]. Recent studies indicate that CHIKV does not activate leukocytes or dendritic cells for the induction of IFNs, but the type-1 IFNs are produced by infected fibroblasts [74]. Clinical studies indicate that the innate immunity efficiently clears circulating CHIKV within 4–7 days [73]. Normally adaptive immune response with activation of specific B and T lymphocytes occurs after a week and likely does not play a role in controlling the acute infection, but the relative role in the chronic phase of the disease is unknown. Generation of CHIKV-specific neutralizing antibodies to protect against reinfection occurs during convalescence, and recovery and prophylactic administration of specific antibodies can protect against infection or disease in mice, but not 24 h after inducing infection [75]. The role of cellular immunity in CHIKV infection is undetermined, but there is likely a beneficial response, as alphavirus-specific cytotoxic T lymphocytes can eliminate persistently infected macrophages with Old World alphaviruses [76]. Many patients recovering from CHIKV infection have chronic joint disease for several months to years, and the mechanism remains unclear. A single report suggests possible induction of autoimmune response by lymphocytes caused by cross-reactivity between viral and host antigens [77]. It has also been suggested that chronic joint symptoms are due to persistence of the virus with reactive inflammation with persistent virus-specific IgM [78], and persistence of viral RNA has been demonstrated in a patient with chronic arthralgia for 18 months after CHIKV infection [79]. Current evidence indicates that CHIKV infection results in lifelong immunity and there is no significant antigenic variation between genotypes to result in immune escape [8].

6.5.2 DENV Disease Pathogenesis

The pathophysiology of DENV infection is not well understood because of the lack of a suitable animal model. Most studies have focused attention on severe dengue disease or the dengue hemorrhagic syndrome [DHS]. Clinical epidemiologic studies have identified several risk factors for severe disease: young age, female sex, high body mass index, virus strain, and genetic variants of the MHC-1-related sequence B and phospholipase C epsilon 1 genes [80]. Reinfection or two sequential infection with different serotypes is linked to severe dengue disease through antibody-dependent enhancement of the immune response, with the infecting virus forming an immune complex that enter Fc receptor-bearing cells [81]. The concept of antibody-dependent enhancement by weakly cross-reactive antibody from another serotype does not explain adequately that in most dengue endemic countries, the populations at large have been exposed to multiple serotypes with cross-reactive antibodies to all four serotypes by age 14 years and above, yet severe dengue syndromes are relatively rare [82–84]. Studies in Thailand found that only 0.5–2% of secondary infection resulted in shock [84]. This suggests that there is a genetic predisposition to

severe dengue syndromes. Another aspect of the hypothesis of severe disease mechanism suggests that high viral load in the blood with infection of endothelial cells results in stimulation of high levels of cytokines and soluble mediators, resulting in vascular fragility and bleeding. However, there is no good evidence to support the correlation of high viral load and severity of disease. Hence, it has been proposed that an aberrant immune response with an imbalance between proinflammatory and anti-inflammatory cytokines results in a cytokine storm [85]. Several cytokines may induce bleeding and increase vascular permeability, through induction of apoptosis [cell death] of platelet precursors and endothelial cells, to produce thrombocytopenia and affect endothelial cell adherent junctions [85].

There is also evidence that the sequence of DENV serotypes infection plays a role in the risk of development of severe dengue syndromes. The highest risk for severe disease has been associated with DENV-1/DENV-2 followed by DENV-1/DENV-3 and lowest for DENV-2/DENV-3 [86]. Studies have found that young children have the highest risk of developing severe disease after a second infection [85] and infants born to dengue-immune mothers are at the greatest risk of severe disease [85]. It was initially opined that risk of severe dengue syndromes would occur only within the first 5 years after primary infection, but attack rates of these syndromes in the same population with primary DENV-1 and secondary DENV-2 were significantly higher 20 years versus 4 years after repeat infection [84]. A recent review and meta-analysis of factors associated with dengue shock syndrome [DSS] confirmed the association of several recognized risk factors, young age, secondary dengue infection, female sex, DENV-2, and several manifestations of neurological disorders and liver damage [the latter two factors likely represent severity of the disease], but there have been a sustained decline in DSS and dengue hemorrhagic fever [DHF] in Southeast Asia for the last 40 years [87]. It is postulated that declining rates of severe dengue disease may be related to educational programs, early rehydration treatment, and the increasing age of the average population.

6.5.2.1 DENV Immunity

Following a mosquito bite with DENV, the virus enters and replicates in Langerhans cells and then migrates to lymphoid tissue for further amplification, and viremia can be detected in 5–7 days with primary infection and only lasts 2–3 days during secondary infection [88]. During primary viremia, the virus infects tissue macrophages of several organs, especially the liver and spleen where the virus may continue to replicate after the cessation of the viremia [88]. The initial innate response to the infection consist of the combined effect of type-1 IFNs, tumor necrosis factor alpha [TNF-α], and cell surface receptor-ligand interactions in stimulating the anti-dengue response of primary human natural killer [NK] cells [89]. The NK cells produce cytokines and lysis of target infected cells. There is also recent evidence that activated and apoptotic platelets aggregate with monocyte and signal-specific cytokine [IL-1β, IL-8, IL-10, and MCP-1] responses that may contribute to the pathogenesis of dengue [90]. During primary infection, IgM antibodies become detectable about

day 5 after onset of symptoms, peaks at day 10, and may last for 6 months [86]. Neutralizing IgG antibodies appear after the first week with primary infection and peak during convalescence, 14 days or after, and provide specific serotype protection for life and cross protection for heterologous virus for at least 2–3 months after infection and for up to 1–3 years [88]. During secondary infection, IgG antibody is produced faster and to higher titers and IgM if produced is diminished and may last for four months.

It is believed that secondary infection of a heterologous virus can induce antibody-dependent enhancement [ADE] of infection. The exact mechanism of ADE is still unclear. It is postulated that cross-reactive non-neutralizing antibodies enhance the uptake of virus by monocytic cells and this results in extreme immune activation that results in cytokine storm [91]. Other hypotheses include that virus-antibody complexes activate the complement pathway to enhanced immune response resulting in cytokine storm or dengue-specific antibodies cross-react with host proteins to activate the coagulation pathway and alter endothelial cell function [91]. There is evidence that CD4+ and CD8+ T-cell activation plays a role in protection against dengue infection and aberrant CD8+ cell response may be involved in the cytokine storm in severe disease [88, 92]. Occasionally severe dengue disease [DHF] can be seen in primary infection in older children and adults [92].

6.5.3 ZIKV Disease Pathogenesis

There is sparse data on the pathogenesis of ZIKV infection, but once the virus enters the human body by a mosquito bite, it infects dermal fibroblasts and dendritic cells through adhesion factors and induces transcription of Toll-like receptor 3 [TLR3] and other molecules, to stimulate several IFN genes and strongly enhance IFNβ gene expression [93]. ZIKV gains entry to skin fibroblasts and immune cells by the phosphatidylserine receptor AXL and upregulates the autophagy pathway, which enhances replication in autophagosomes [93]. There is evidence that flavivirus replication and pathogenesis involve several cellular pathways such as endoplasmic reticulum stress, cellular signaling response termed unfolded protein response, and autophagy [94]. Limited studies in patients had demonstrated polyfunctional T-cell activation, Th1, Th2, Th9, and Th17 response, during the acute phase with respective cytokine level increases, followed by a decrease in the recovery phase [95]. ZIKV is sensitive to the antiviral effects of both type-1 and type-2 IFNs [95], which likely result in clearance of the virus. However, flaviviruses in general surmount IFN type 1 signaling in order to proliferate intracellularly. ZIKV accomplish this by degradation of the STAT2 signaling molecule downstream of the IFN receptor in human cells [96].

In utero transmission and congenital abnormalities from ZIKV infection have attained global attention since the large epidemic in Brazil and the Americas, such as microcephaly, spontaneous abortion, and intrauterine growth restriction. Recently, four studies using the mouse pregnant model have investigated the effect of ZIKV on the fetuses [97–100]. Inoculation of different isolates of ZIKV was via peripheral

routes or directly into the fetal brain, and all studies demonstrated infection of the fetuses with brain cell damage. One of the studies [97], using deficient-type IFN-signaling mouse model and cutaneous inoculation of an Asian ZIKV strain in the first trimester [human equivalent], showed placental infection, injury, and impairment, with fetal brain injury and neuronal cell death. These findings would explain the human experience with ZIKV in the first and second trimester of pregnant women. These studies indicate that ZIKV can cross the placental barrier to gain access to the fetus and infect placental cells and fetal endothelial cells to cause vascular damage and impair growth and development. Moreover, seeding to the fetal brain can injure or cause death of neuroprogenitor cells and inhibit cell differentiation to explain the cortical thinning, microcephaly, and brain structure abnormalities in neonates of infected pregnant women [101]. Although ZIKV has been documented in maternal blood 5 days after acute symptoms, it also has been rarely reported 8 weeks and even up to 107 days after the onset of symptoms, suggesting persisting viral replication in the fetus or placenta [102].

6.5.4 ZIKV Immunity

Most ZIKV infections are asymptomatic. Viremia is believed to occur from several days before onset of illness to a week after illness and has the potential for transmission by blood transfusion [103]. ZIKV-specific IgM antibodies develop during the first week of illness, but data on duration are limited [33]. IgG neutralizing antibodies develop shortly after IgM antibodies and probably persist for many years after infection to confer prolonged and possibly lifelong immunity [104]. However, this presumption is largely based on data from other related flaviviruses such as West Nile virus and yellow fever virus. There is no known antigenic variation of different genotypes; thus, immunity should be cross protective for isolates from different regions of origin, as there is no strain subtypes of ZIKV.

6.6 Clinical Manifestations

6.6.1 CHIKV Clinical Disease

The acute manifestation of CHIKV infection is abrupt with high fever, chills, headaches, myalgia, and arthralgia and clinically indistinguishable from dengue fever. The incubation period ranges from 1 to 12 days but averages 2–4 days, but unlike dengue asymptomatic infection is very uncommon, about 3–25% of people with serological evidence of infection [8]. A maculopapular, erythematous rash on the trunk with possible involvement of the face, limbs, palms, and soles can be present in 20–80% of patients and is rarely bullous in children [10]. Severe polyarthralgia, which can be disabling, is the hallmark of this disease, and debilitating

polyarthralgia in endemic countries even with circulating DENV have a positive predictive value of 80% for CHIKV viremia [8, 105]. The arthralgia in dengue fever is usually milder and of shorter duration during first few days with fever. In CHIKV infection, polyarthralgia is usually symmetrical and involves joints of the arms and lower limbs [about 90%] and less often the spine, and large joints and small joints are frequently affected [8, 10]. Joints with previous damage from other disease such as osteoarthritis are often more severely affected with disabling arthritis. Swelling and periarticular edema may be seen especially in the interphalangeal joints, wrists, and ankles and represent active arthritis, but joint effusions have not been a notable feature. Nonspecific symptoms of vomiting, diarrhea, abdominal pain, confusion, and weakness have been reported in large outbreaks of hospital referred patients [8].

Although CHIKV is not considered a neurotropic virus, various neurological complications have been reported in multiple large outbreaks from India and the Indian Ocean Islands, both in adults requiring hospitalization and in young children and neonates with mother-to-child in utero transmission [8, 106]. In adults requiring admission to hospital in these outbreaks, neurological manifestations have been reported in 15–25% of cases, and these include encephalopathy, seizures, encephalitis, Guillain-Barre syndrome, and encephalomyelitis [72]. Neurological signs were less frequent and severe in hospitalized children but also included seizures, encephalitis, meningeal symptoms, and acute encephalopathy. Infected neonates were especially prone to CNS complications with 50% showing abnormalities on magnetic resonance imaging of the brain including white matter lesions, cerebral hemorrhage, and edema, which may lead to death and severe disabilities in 10–20% [72]. Autopsy of infected neonates and murine model of CHIKV infection have demonstrated brain pathological changes from the infection. Death rate following CHIKV infection was estimated to be 1:1000 cases in La Reunion outbreak [106] but is usually reported rarely in other outbreaks, and mortality is generally associated with multiple comorbid illness in the elderly or neonatal infection. These complicated cases may be associated with rare complications such as hemorrhage, liver dysfunction, renal failure, heart failure, and myocarditis [8, 10, 66]. Other rare manifestations of CHIKV infections include conjunctivitis, uveitis, retinitis, iritis, and chondritis of the ear [10].

Most patients improve 1–2 weeks after the onset of acute illness, but a high proportion of patients have persistent arthralgia from several months to years. In a study from the Reunion Island outbreak, 57% of infected patients had persistent or recurrent polyarthralgia after 15 months [107]. In this study and others, persistent arthralgia was related to age and being greater in those older than 45 years of age. Even 3 years after acute infection of CHIKV 12% of patients may still have residual joint pain, stiffness, and swelling [108]. Chronic arthralgia is more common in the distal joints and may mimic rheumatoid arthritis, with increased inflammatory markers and erosive changes on imaging [rarely deforming polyarthritis] in up to 50% of patients followed for 36 months [109]. Patients with joint pains up to 36% met the criteria for rheumatoid arthritis but with negative rheumatoid factor, and MRI findings may include joint effusion, bony erosions, marrow edema, synovial thickening, tendinitis, and tenosynovitis [110]. Treatment of acute and chronic

arthralgia of CHIKV infection is primarily the use of nonsteroidal anti-inflammatory agents [NSAIDs], and although methotrexate has been used for more disabling arthritis, there is no clinical trial with immunosuppressive agents [111].

CHIKV infection is most often confirmed by serological methods. Indirect immunofluorescence and ELISA tests are sensitive and rapid techniques for diagnosis and differentiate the presence of IgM or IgG antibodies [8]. Specific IgM antibody can be detected between 2 and 7 days after onset of illness by ELISA and immunofluorescence and even after 1 day by lateral-flow rapid test. IgG antibodies are most often detected after 5–7 days and occasionally after 2 days. IgM antibody persists for 3–4 months, and reports of persistent IgM after 2 years may suggest persistent virus in the joints [78]. IgG antibody specific for CHIKV persists for many years. CHIKV can be isolated on mammalian cell cultures such as Vero cells, and RT-PCR can be used to detect the virus from 1 to 7 days after onset of symptoms [8]. Antigen capture ELISA technique has also been used to detect the virus in serum and cerebrospinal fluid after 2 days of illness [10].

6.6.2 DENV Disease

Most patients infected with the DENV in endemic regions are asymptomatic or experience minimal symptoms [112], a major difference from CHIKV infection. The incubation period after inoculation is 3–7 days and starts suddenly, and the illness is divided into three phases: an acute febrile phase, a critical phase around the time of defervescence, and a recovery phase [80]. The initial phase is usually heralded by high fever, headaches, retro-orbital pain, myalgia, arthralgia, nausea and vomiting, and sometimes a transient macular rash [80, 88]. Mild bleeding, bruising, and petechiae may be evident and an enlarged liver may be palpable. Although children have high fever, the other symptoms may be less severe than adults in this phase. Common laboratory abnormalities often include mild to moderate leukopenia, thrombocytopenia, and liver enzyme elevations, similar to disturbances that can be seen with CHIKV infection. Majority of patients recover without complications after 3–7 days.

A small proportion of susceptible patients, mainly children and young adults, may progress to the critical phase at the time of resolving fever [80, 88]. This stage of illness is characterized by worsening symptoms of weakness with systemic signs attributable to increased vascular permeability and leaky vessels. These consist of signs of hypotension and shock, associated with worsening and persistent vomiting, abdominal pain with tender hepatomegaly, development of pleural and peritoneal effusions, and subsequently bleeding from the mucosa, restlessness, and lethargy. Failure to institute adequate medical therapy may result in refractory shock, severe gastrointestinal bleeding, hepatorenal failure, encephalopathy, rarely myocarditis, and death. Results of laboratory tests at this phase usually reveal moderate to severe thrombocytopenia, with platelet counts often below 20×10^{-9} per liter, and increase in activated partial-thromboplastin time, and low fibrinogen may exhibit levels resembling but not typical for disseminated intravascular coagulopathy [80].

Up to two-thirds of patients may have hypokalemia, and hypokalemic paralysis is an underemphasized neuromuscular complication of severe DENV infection [113]. Elevated creatine kinase from rhabdomyolysis is seen occasionally [114], and acute kidney failure occurs in 2–5% of patients and carries a high mortality [115]. In a recent study of 796 pediatric patients, the most common findings included thrombocytopenia [96%], abdominal pain [71%], and vomiting [59%], but the most important factors associated with severe dengue were rash, severe thrombocytopenia, and anemia [116]. This critical phase is short-lived and lasts for 48–72 h with subsequent rapid recovery in the recovery phase in most cases. The mortality in patients with refractory shock, usually from late medical attention, can be up to 20% but with early appropriate treatment is usually very low, from 0.1% to 0.5% [88]. The critical phase is characterized by very low viremia, peak T-cell activation, and vasculopathy. Current evidence suggests that platelet and complement activation through antibody-mediated immune complex results in the combined effect of anaphylatoxins and inflammatory molecules and platelet sequestration results in vasculopathy [117]. Since 2009, the World Health Organization has replaced the 1997 classification of dengue/dengue hemorrhagic fever/dengue shock syndrome with dengue and severe dengue [118].

During the recovery phase, there is gradual improvement of any organ dysfunction, and a second mild maculopapular rash may appear that eventually resolves in 1–2 weeks, but fatigue may persist in adults for several weeks [80]. Unlike CHIKV infection, chronic sequelae from dengue have been unrecognized. However, neurological complications such as encephalitis, myositis, myelitis, Guillain-Barre syndrome, and neuropathies can occur with long-lasting effect [119, 120].

Laboratory diagnosis of dengue is usually accomplished by serological methods. IgM antibodies can be detected as early as 4 days after onset of fever by ELISA or lateral-flow rapid test [80]. Although IgM antibodies from a single specimen provide a presumptive diagnosis, seroconversion from paired sample is confirmatory. In secondary dengue infection, dengue-specific IgG antibodies may predominate over IgM due to the rapid anamnestic antibody response. In the initial febrile phase rapid, early diagnosis can be made by detection of DENV RNA by RT-PCR or detection of the virus-expressed soluble nonstructural protein 1 [NS1] by ELISA or lateral-flow rapid test [80]. In primary infection during the febrile phase, detection of NS1 antigen is >90% but is 60–80% in secondary infection [121, 122]. Antigenemia may persist for several days after resolution of the fever, and combined antigen and antibody testing improves the diagnostic sensitivity [123].

Management of dengue fever is largely symptomatic and supportive as with CHIKV infection. Mild to moderate disease can be managed with NSAIDS for pain control, but aspirin is best avoided because of increased risk of bleeding. Severe disease requires prompt fluid and electrolyte disturbance correction with intravenous crystalloids and oral replacement therapy. Blood and platelet transfusions may be required for bleeding complications, but there is a lack of guidelines for transfusion support in patients with severe dengue and no studies on the benefit of prophylactic platelet transfusion [124]. Patients in shock may require vasopressors to support an adequate blood pressure, hemodialysis for those in renal failure, treatment for heart

Table 6.1 Comparison of CHIKV, ZIKV, and DENV infections

	CHIKV	DENV	ZIKV
Virology	*Togaviridae*	*Flaviviridae*	*Flaviviridae*
Virus	Enveloped, single-stranded RNA	Same	Same
Origin	Africa	Africa or Asia	Africa
Strains	1 strain, 3 genotypes	4–5 strains	1 strain, 2 lineages
Vector	*Aedes aegypti, Ae. albopictus*	Same	Same
Distribution	Tropical and subtropical globally	Same	Africa, SE Asia, Americas, Pacific
Clinical aspects			
Incubation period	2–4 days	3–7 days	3–12 days
Subclinical infection	Rare [3–<20%]	Common	80%
Symptoms: acute	Severe polyarthralgia	Flu-like illness, bruising	Mild flu-like illness
Duration of acute	7–14 days	5–7 days	3–5 days
Severe disease	Very rare [neonates]	Children [2%]	Rare Guillain-Barre syndrome
Critical phase	None	Shock and bleeding [48–72 h]	None
Recovery phase	None	7–14 days	None
Chronic disease	Arthralgia/arthritis [12–50% at 3 years]	None	None
Congenital	Rare CNS disorder	None	Microcephaly
Treatment	NSAIDS	NSAIDS, blood and platelets	Acetaminophen, NSAIDS
Mortality	<1: 1000	Severe dengue—0.2–20%	Stillbirth [rare]
Promising drug	None	Prochlorperazine at onset	None
Vaccine	None	Dengvaxia approved in Mexico	None

failure in subjects with myocarditis and fluid overload, and mechanical ventilation to manage ARDS and comatose patients. Table 6.1 shows a comparison of the clinical and virological features of DENV, ZIKV and CHIKV infections.

6.6.3 ZIKV Disease

Prior to the Yap Island outbreak of 2007, ZIKV caused only sporadic infections with mild flu-like illness resembling a mild form of dengue fever in Africa and Southeast Asia. Thus cases of ZIKV infections were probably misdiagnosed and under recognized and under reported. In the Yap Island outbreak, it was estimated that 73% of the population were infected, but most patients were asymptomatic [about 80%],

and clinical symptoms were mild with mainly fever, rash, arthralgia, myalgia, and conjunctivitis [125]. Although *Aedes hensilli* was the predominant mosquito circulating at the time, the virus was never detected in this species. A large epidemic of ZIKV occurred in French Polynesia in 2013–2014 before spreading throughout the Pacific region [126]. This unusual epidemic was unprecedented as there were concurrent outbreaks of DENV, CHIKV, and ZIKV around the same time [127]. In the French Polynesia, there were reports of increased severe neurological complications [Guillain-Barre syndrome] first time associated with ZIKV [127], but possibly related to concomitant infection with CHIKV or DENV. The Polynesia outbreak occurred rapidly soon after the index case, with 28,000 cases of ZIKV infection in the first four months and 42 cases of Guillain-Barre syndrome [44]. Based on phylogenetic analysis, the ZIKV introduction to Polynesia was independent of the Yap Island outbreak and came separately from Southeast Asia. [44]. Transmission to the Americas appears to have originated from the Pacific islands.

ZIKV spread from the South Pacific islands to South America by February 2014, where it was first recognized to be locally transmitted on Easter Island off the coast of Chile, and locally transmitted cases appeared in May 2015, in the northeastern region of Brazil [WHO; Weekly epidemiological record. November 6, 2015. http://www.who.int/wer]. By December 2015, there were 440,000–1,300,000 cases of ZIKV infection in Brazil with a 20-fold increase in infants born with microcephaly and with detection of the virus RNA in the amniotic fluid of affected newborns [128]. Microcephaly has never been associated with previous ZIKV outbreaks, but the virus was detected in tissues of a baby with microcephaly that died shortly after birth [129]. Lack of standardization of the definition of microcephaly may have affected the reporting of this condition, but CDC has recommended a definition of an occipitofrontal circumference below the third percentile for age and sex [130]. Microcephaly can lead to seizures, developmental delays, learning disabilities, and impaired motor function. Infections associated with microcephaly would usually occur during the early weeks of fetal development to result in loss of brain cells in the developing brain. However, microcephaly can result from fetal brain disruption sequence, with normal brain development in early pregnancy and subsequent collapse of the skull with fetal brain destruction [131]. Reports of infants with microcephaly related to ZIKV infection in Brazil are consistent with fetal brain disruption, and there is some evidence that this could occur even in the second or third trimester of pregnancy, although the greatest risk is in the first trimester [132]. Preliminary report from Brazil has noted the presence of fetal abnormalities in 29% of pregnant women infected with ZIKV on ultrasonography [133]. There is increased fetal death with infection between 6 and 32 weeks of gestation [133]. The possible explanations for this newly recognized complication of ZIKV infection are the following: (1) previous outbreaks were too small to detect a rare complication; (2) the virus may have mutated to become more neurovirulent; and (3) synergistic effect of coinfection with another circulating virus such as CHIKV or DENV. However, a recent retrospective study of the French Polynesian outbreak in 2013–2015 suggests that the association of microcephaly and ZIKV infection was missed but is evident on recent analysis [134]. In this study, the baseline prevalence of microcephaly was two cases per 10,000 neonates, and the risk associated with ZIKV infection was 95 cases

per 10,000 women infected in the first trimester. Other neurological complications of ZIKV recently reported include meningoencephalitis, acute myelitis, and optic abnormalities [especially in neonates with or without microcephaly] [135, 136].

Diagnosis of ZIKV infection is often assessed by serological methods such as ELISA for IgM or IgG antibodies, which can be difficult to interpret due to cross-reaction with other flavivirus such as DENV or West Nile virus, and antibodies may not be present in early infection. Fetal cord blood at birth with ZIKV-IgM would be specific as IgM would not cross the placenta barrier. Antibodies appear about 5–6 days after onset of symptoms, and confirmation of a positive serology may require the ZIKV plaque neutralization test [PRNT], which is laborious and time consuming [137]. For the acute diagnosis with symptoms less than 10 days, serology and RT-PCR should be performed. A one-step rRT-PCR appears to be very sensitive and specific [138]. ZIKV is present in the blood for the first 3–5 days of illness but can be detected in urine >10 days after onset of illness [139], and in saliva even when the blood was negative by RT-PCR [140].

There is no specific treatment for ZIKV infection and only symptomatic or supportive therapy is available. For pregnant female with headaches or pain, acetaminophen can be used; otherwise, aspirin or NSAIDS may be used when dengue fever is not likely. Serial ultrasounds every 3–4 weeks are recommended in pregnant women with confirmed or suspected ZIKV infection. Fetal abnormalities may be detected by 18–20 weeks of gestation but usually later and may include abnormalities other than microcephaly such as intrauterine growth retardation, hydrops fetalis, anhydramnios, cerebral calcification, hydrocephalus, brain atrophy, absent corpus callosum, and hydranencephaly [141]. For suspected congenital infection amniocentesis and testing by RT-PCR could be considered with appropriate counseling of the parents of available options. At birth testing of the infant serum or umbilical cord blood, and cerebrospinal fluid, should be tested for ZIKV by serology and RT-PCR, plus PCR of the placenta [137]. Neurological assessment including head ultrasound, ophthalmologic examination, and hearing evaluation should be performed and neurological monitoring throughout infancy to assess for long-term sequelae. A recent report documented ocular abnormalities in infants with microcephaly presumably related to intrauterine ZIKV infection [142]. These findings included macular alterations, gross pigment mottling or choreoretinal atrophy, and optic nerve abnormalities in a high percentage [35%] of affected infants [143].

6.6.3.1 Congenital ZIKV Syndrome

The largest and most comprehensive study on congenital ZIKV syndrome, including microcephaly, was recently published based on data from the Brazilian ministry of health surveillance system for microcephaly. A total of 7830 suspected cases were reported to the Brazilian Ministry of Health by June 2016. Among a total of 5554 live-born infants with suspected microcephaly, investigation was completed on 1501 suspected cases [27%], of which 602 [40%] were considered definite or probable cases of congenital ZIKV neurological disorder [based on head circumference for gestational age below 2SD and specific neuroimaging findings [141]]. Rashes in the

third trimester of pregnancy were associated with brain abnormalities despite normal head size [present in about 20% of definite or probable cases], but rash during pregnancy did not occur in a third of the affected cases. Distinctive neuroimaging findings included brain calcifications, ventricular enlargement, or both with negative serology for syphilis, toxoplasmosis, and cytomegalovirus; however, some of the probable cases had incomplete data to exclude these other congenital infections. Also reported at the same time is a case series [$N = 5$] of the pathological findings of congenital ZIKV syndrome [144]. In all three fatal cases with microcephaly, [two also had severe arthrogryposis] viral antigens were localized to glial cells and neurons and associated with microcalcification. ZIKV antigens were detected in chorionic villi of one of the first trimester placentas [with spontaneous abortion], and tissue samples from all five cases were positive for ZIKV RNA by RT-PCR [144]. Thus, these findings provide strong evidence for ZIKV causing congenital central nervous system malformations, including microcephaly, arthrogryposis [flexion contracture of the limb/joint], and spontaneous abortion.

6.7 Potential Treatments and Vaccines

6.7.1 CHIKV Treatment

Although in vitro studies show that IFN-α have activity against CHIKV and combined with ribavirin there is synergistic antiviral effect [145], they are unlikely to have a significant role in future treatment. The major debilitation is from chronic severe arthralgia or arthritis, and novel or existent therapies are needed. Chloroquine or its derivative hydroxychloroquine have anti-inflammatory properties and have been used for rheumatological diseases for decades. However, clinical studies with chloroquine in CHIKV infection have produced mixed results. Early, small open clinical trial suggested that chloroquine might be beneficial for chronic arthralgia after CHIKV infection, but a later study of 27 patients with acute CHIKV treated with chloroquine compared to 27 controls showed no benefit and increased incidence of chronic arthralgia [146, 147]. Administration of specific CHIKV immunoglobulins harvested from recovered patients has high neutralizing activity and has been shown to have protective and therapeutic activity [within 24 h after inoculation] in the mouse model [75]. However, the potential use in an outbreak would be limited to specific high-risk scenarios such as neonates born to viremic mothers or adults with underlying rheumatological conditions such as rheumatoid arthritis. Development of an effective CHIKV vaccine should be simpler than producing an effective DENV vaccine because of limited antigenic variation among genotypes and lack of antibody enhancement. Although several vaccines in phase 1 or preclinical studies are in development [148], marketing a commercially viable vaccine will be challenging. CHIKV outbreaks have been unpredictable, and to perform a phase 3 clinical trial would be difficult as disease surveillance declines after epidemics. Usually afflicted population would have long-lasting protection, and new outbreaks require a susceptible population at risk.

6.7.2 DENV Vaccines

Development of an effective and safe DENV vaccine has been a major goal and priority by the scientific and research community for over two decades. The major concern of vaccine-induced disease from antibody enhancement has not been realized from vaccines that have been tested to date, likely from development of tetravalent vaccines to provide antibodies to all serotypes at the same time. Several DENV vaccines are in development at various stages, but the Sanofi Pasteur vaccine [Dengvaxia], developed over 20 years and the first dengue vaccine to be marketed and approved in Mexico in December 2015, for an initial phase with 40,000 people [children] to be inoculated. [http://www.sanofipasteur.com/en/articles/dengvaxia-world-s-first-dengue-vaccine-approved-in-mexico.aspx]. The vaccine is a recombinant, live-attenuated, tetravalent dengue vaccine [CYD-TDV] composed of four chimeric live flaviviruses, each derived from the yellow fever virus genome with gene segments of each of the four DENV serotypes [142]. The vaccine was given to >35,000 children between the ages of 2 and 16 years in Asian-Pacific and Latin American countries as three subcutaneous injections given at months 0, 6, and 12 and assessed in three clinical trials [148–151]. The vaccine efficacy after a 3 year assessment was shown to reduce dengue due to all four serotypes in nearly two-thirds of participants, and pooled analysis showed the vaccine prevented 9 out of 10 cases of severe dengue and 8 out of 10 hospitalizations in ages 9–16 years, but there was an unexplained higher incidence of hospitalization in children younger than 9 years of age [151]. This latter observation has to be carefully monitored during long-term follow-up to assess for antibody enhancement-induced disease. The vaccine was found to be safe and serious adverse event in the first 28 days of vaccination in the vaccine and control group was similar [each 1%] [151].

There are several drawbacks of the CYD-TDV vaccine including the need for multiple dosing; modest protective efficacy of only 60% overall at 3 years, with even lower efficacy [35.5%] in flavivirus-naïve subjects [150, 151]; and evidence of unequal and waning immunogenicity long after the third dose of the vaccine [152]. Another potential concern would be development of severe dengue disease in vaccine recipients much later in life, a trend which is already appearing in the pre-vaccine era.

Currently there is no effective agent for treatment of dengue. Chloroquine, a cheap and widely available drug, has modest in vitro activity against DENV, but a randomized controlled trial failed to show any clinical benefit [153].

6.7.3 ZIKV Vaccine

Research in developing an effective vaccine for ZIKV is occurring at a rapid unprecedented pace. Preliminary studies of a purified inactivated ZIKV [PIV] vaccine and a DNA vaccine expressing an optimized premembrane and envelope [prM-Env] immunogen showed protective efficacy in mice challenged with both ZIKV strains from Brazil and Puerto Rico [154]. Further studies by the same investigators have recently shown that three different vaccine platforms protect against ZIKV challenge in rhesus

monkeys [155]. The purified inactivated virus vaccine induced ZIKV-specific neutralizing antibodies and completely protected against ZIKV challenge with strains from both Brazil and Puerto Rico. In addition, adoptive transfer studies demonstrated that purified immunoglobulin from vaccinated monkeys conferred passive protection. A plasmid DNA vaccine and a single-shot recombinant rhesus adenovirus vector expressing ZIKV prM-Env also completely protected monkeys against ZIKV challenge. Hence, rapid clinical development of a ZIKV vaccine for humans is a reality.

6.8 Future Prospects

The most vexing challenge which would provide the most cost-effective benefit against CHIKV, ZIKV, and DENV infections is effective vector control. Although simple measures have been available to communities for many decades to decrease the risk of mosquito bites, they are poorly adhered to over an indefinite period. Two novel approaches to control the mosquito population appear to be of some benefit from preliminary studies. The release of genetically modified male mosquitoes that sterilize the circulating female population to reduce egg output and subsequent mosquito population had been shown to be feasible in a localized region such as a small island [156]. However, it is difficult to envision that this technique could be highly effective in large continents in Asia, Africa, and the Americas with numerous adjoining countries. An alternative strategy is the induction of widespread biologic resistance to DENV and CHIKV in *Aedes* mosquito population. *Wolbachia* species are bacterial endosymbionts of insects, helminths, and crustaceans that are transmitted by transovarial and transstadial [between stages of development passage] means and are important in the pathogenesis of filariasis [157]. *Wolbachia*-infected *Ae. aegypti* are partially resistant to DENV and CHIKV infections [158] and the bacteria can invade and establish infection in *Ae. aegypti* populations [158, 159]. Wolbachia infected mosquitoes are also highly resistant to ZIKV [Duttra et al., 2016; Cell Host & Microbe; 19: 771–4]. Unlike the use of chemical insecticides, there would be minimal risk to humans and less chance of resistant mutants developing. *Wolbachia* species are nonpathogenic to humans [160]. Laboratory reared male mosquitoes infected with Wolbachia when released in the environment mate with multiple females, rendering them infertile and eventually reduce the pest population. Preliminary studies in targeted areas in Australia, Indonesia, Vietnam, and China indicate that this is feasible and 90% of local mosquitoes can become infected within weeks. Plans are in progress to widely release Wolbachia infected mosquitoes to fight ZIKV in Rio de Janeiro and Medellin [Columbia] over the next 2 years [161]. The USA is reviewing and considering similar strategy and a biotechnology firm [MosquitoMate] is seeking approval to market Wolbachia infected mosquitoes as a pesticide [http://www.nature.com/news/us-reviews-plan-to-infect-to-stop–12/19/2016].

The most promising of the dengue vaccines in development [TV005] is a live-attenuated tetravalent vaccine designed by the Laboratory of Infectious Diseases at

the National Institutes of Health [Bethesda, Maryland]. All the vaccine components have a DENV genetic background and share a core attenuating, 30-nucleotide deletion in the viral genome of each strain, yielding replication-deficient attenuated viruses [162]. The precursor vaccine [TV003] DENV-2 component was less immunogenic than other serotypes, but the TV005 vaccine contained a highe-dose component of DENV-2. A recent preclinical study has shown that a single subcutaneous injection of the TV005 vaccine elicited a tetravalent response in 90% of vaccines at 3 months after vaccination and a trivalent response in 98% [163]. The vaccine was well tolerated. Further studies such as a phase 3 clinical trial in a large at-risk population may take several years to accomplish but are eagerly awaited.

Development of vaccines for CHIKV is feasible and in the very early stages. Two live-attenuated CHIKV vaccine candidates have been shown to be safe and effective in preventing viremia and clinical disease in nonhuman primates after a single dose [164]. It is also possible to develop a DNA vaccine to initiate replication of live vaccine CHIKV in vitro and in vivo that can elicit neutralizing antibodies and protect mice from a neurovirulent CHIKV [165]. The main challenges facing development of an effective CHIKV vaccine will be completion of a large phase 3 clinical trial, as disease outbreaks are sporadic and unpredictable, and to have a viable commercial market to make it cost-effective.

Drug development of antiviral agents for DENV and CHIKV is in its infancy, and progress toward drug candidates has been very slow. A not so novel but practical approach and cost efficient is the repurposing of existent marketed drugs for a new indication. Prochlorperazine [PCZ] is readily available and approved for treatment of nausea and vomiting as a dopamine D2 receptor antagonist. PCZ can block DENV infection by targeting viral binding and viral entry through D2R, and clathrin-associated mechanisms and administration soon after infection can protect against lethality in Stat1-deficient mouse model [166]. Thus, prophylactic and early administration in the acute, febrile phase of dengue preclinical studies of this drug would be unnecessary and may improve clinical symptoms and outcome. Hence, a large randomized, controlled, clinical trial is warranted with PCZ for any new outbreak of dengue, as further preclinical studies of this drug would be unnecessary.

ZIKV vaccine development is being explored by pharmaceuticals, and the US government has promised to invest hundreds of millions of dollars toward an effective vaccine. The obvious candidates for an effective ZIKV vaccine would be uninfected pregnant women or women of child-bearing age in an outbreak. A major challenge is to conduct a phase three efficacy trial before termination of any ongoing outbreak. While separate vaccines for DENV,CHIKV, and ZIKV is the first step for effective prevention of these emerging zoonoses, a multiple virus vaccine for all three viruses should be explored in experimental animal models. Further research is needed to unravel the mechanism of ZIKV infection and microcephaly and to explore potential preventative measures in pregnant nonhuman primates. Another laboratory model to study microcephaly is being initiated by scientists in Brazil by infecting cerebral organoids with ZIKV. Cerebral organoids are tiny models of the human brain grown from stem cells in laboratory dish that already has been used to study the mechanisms of genetic mutations causing microcephaly in Austria [167].

Perhaps more research and funding would be better served to investigate development of effective means of eradicating or controlling *Aedes* mosquitoes or explore the

potential of a vaccine to protect against mosquito bites. This would be one solution to protect against four emerging viruses of public health concern, as yellow fever virus is transmitted by the same vector. Currently, the fight against the vector in Brazil and other affected countries includes a combination of education of the public to reduce mosquito bites [protective clothing, screened doors and windows, insect repellant with DEET], methods to discourage breeding sites in and around the home [such as discarding empty containers and old tires] or use of larvicides for collected ponds of water, and insecticide spraying in and around the home. There is little evidence to support the efficacy of various mosquito abatement programs, but measures used in most countries have been of temporary benefit. Thus, it is reasonable for governments to employ a combination of methods: behavioral education campaigns to manage water containers to reduce breeding sites, biological methods such as predatory organisms or bacteria [i.e., *Wolbachia* species] and genetic methods to reduce the population by sterilizing male mosquitoes, and chemical control techniques [insecticide sprays, larvicides, cleaning water containers with household chemicals, or adding paraffin to standing water].

Novel vector control measures are being explored to reduce the mosquito density in local communities as conventional measures often fail. The CDC developed an autocidal gravid ovitrap [AGO] to attract and capture female *Ae. aegypti* mosquitoes responsible for disease transmission. The AGO trap uses wet hay as attractant, is of low cost with no pesticides, and can be used for extended period. In a recent study in Puerto Rico, communities using the AGO traps had tenfold lower mosquito densities than nonintervention communities. Moreover, the proportion of chikungunya virus infection in the intervention communities was one-half that of nonintervention communities [168]. Insectivorous bats may serve as an alternative approach to achieve mosquito control in communities. Under laboratory conditions, bats can consume up to 600 mosquitoes per hour, and a 32% reduction in oviposition [egg deposits] by *Culex* spp. with bat predation had been observed [169].

Addendum Two recent studies have provided new insights on the incidence of congenital abnormalities in maternal ZIKV infection. In a US study of 442 completed pregnancies with recent ZIKV infection, birth defects were identified in 6% and microcephaly in 4% of live births. Birth defects were higher [11%] in first trimester infection with ZIKV [170]. Similar findings were reported from a Brazilian study, with microcephaly in 4 of 117 [3.4%] live births and adverse outcome in 55% of first trimester infection and 29% after the third trimester [171].

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