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Zika virus: lessons learned in Brazil

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Abstract

Zika virus (ZIKV) caused a great commotion in the international scientific community in the last two years due to its association with microcephaly and other neonatal alterations. This review will discuss lessons learned from viral pathogenesis, epidemiology and clinical findings observed during the ZIKV outbreak occurred between 2014 and 2016 in Brazil.

Keywords: Zika virus, epidemics setting, transmission, pathogenesis, arboviruses, Brazil

1. Introduction

Zika virus (ZIKV) was first described as a human pathogen in 1952. ZIKV caused several outbreaks in the African and Asian territories, mainly associated with milder symptoms than those usually observed for other common arboviruses [1]. It was not until 2014 that infection by ZIKV was considered a major public health threat. During the ZIKV outbreak in French Polynesia in 2013 only one report indicated an association between ZIKV infection and neurological disorders [2]. In Brazil a subsequent ZIKV outbreak led to more than two hundred thousand probable cases which quickly spread to other countries in the Americas causing great commotion in the scientific community as an example of the potential of arbovirus (Arthropod-borne Virus) to cause worldwide pandemics [3]. Moreover, unparalleled severe clinical outcomes were also observed at higher ratios than anywhere else. The introduction of ZIKV in Brazil and the correlation of this virus with neurological disorders and microcephaly in neonates from ZIKV-infected mothers, a manifestation known as congenital zika virus syndrome, evidenced several problems regarding diagnosis and treatment as well as assistance to ZIKV-infected patients in a big middle-income country such as Brazil. This review will discuss the latest ZIKV findings focusing on regional characteristics and the challenges during the viral outbreak in Brazil, the most affected country by the congenital zika syndrome, between 2014 and 2016,[3].

2. Brazil: ZIKV epidemics setting

Two years have passed since Brazil was news headlines worldwide due to the ZIKV epidemics. The association between the mosquitos, the virus and its clinical manifestations seem clear and straightforward today. However, back then it was a great challenge that was approached as a joint effort between physicians (obstetricians, clinicians, radiologists, pediatricians, pathologists and others), researchers (basic and clinical research working together), public health and government authorities in Brazil as well as internationally. It all began with individual observations of higher numbers of cases of fever, exanthema and pruritus, Guillain-Barré syndrome, and further increased rates of fetal malformations and microcephaly cases, the final event that certainly called attention towards this disease as an emerging threat.

To address the burden of such epidemic it is essential to understand that although Brazil is a middle-income country with recent economic growth and important improvements in social, environmental and health issues, its continental size associated with extensive regional and social inequalities plays a major role in implementation of effective health strategies. In 1988, Brazil substantially modified its health system with the establishment of a Unified National Health System (*Sistema Único de Saúde*; SUS), which considers the principles of health as a citizen's right and the state's responsibility. SUS aims to deliver universal, comprehensive, preventive and curative care through decentralized management of health services, with community involvement at all administrative levels; financially supported by taxes and social contributions. Coexisting with SUS, there are also two other health delivery systems: Supplementary Health System (SHS) and Private Health System (PHS), covering a minority of the population (around 35 million versus 175 million by SUS) [4].

Approximately two decades later, in response to the challenge of bringing together health research agendas and public health policies and the need to direct more financial resources towards research priorities, the Ministry of Health proposed a National Policy for Science, Technology, and Innovation in Health. This was a long and structured process with the clear intention of defining priorities for all social sectors in order to achieve an increasing equity in the health system [5]. The national policy and the priority agenda are currently regulating investments by the Ministry of Health for research and development and have collaborated with other funding agencies in defining different proposal calls and emergency actions in response to threats such as ZIKV. In this scenario, the zika epidemics emerged in one of the most deprived regions of Brazil, the Northeast [6]. The social impact in this region has been considerable, and is thought to be the cause of a reduction in the number of births registered in 2016, which was reported as 10% in the most affected state, Pernambuco (IBGE data).

There are two ZIKV lineages described (African and the Asian). Sequencing data revealed that only Asian ZIKV was found in Brazil more than 12 months before the first ZIKV case was detected [7]. Those estimations were approximate, but consistent with recent findings suggesting that ZIKV was already circulating in Rio de Janeiro state by April 2013 [8].

In the subsequent months after ZIKV probable introduction in Brazil, it rapidly spread throughout the country causing a massive outbreak (Fig. 1). Autochthonous transmission of ZIKV was confirmed in all federative units of Brazil until mid-2016 [9]. Large-scale sequencing data allow us to infer that the Northeast of Brazil was the epicenter of the ZIKV outbreak, with the virus later spreading to the Southeast and then to other regions [9]. The highest zika virus incidence rate was observed in the states

105 located in the Northeast and Central-West region of Brazil, including Mato Grosso state
106 (714 cases per 100,000 population), Bahia (349 cases per 100,000), Alagoas (208 cases
107 per 100,000), Tocantins (208 cases per 100,000), and Goiás (204 cases per 100,000),
108 and the only state in the Southeast Rio de Janeiro (419 cases per 100,000) [3].

109 Regional characteristics of Brazil, including vaccination against Yellow Fever
110 Virus (YFV) and the circulation of others arthropod-borne viruses with zika
111 overlapping symptoms (such as dengue, chikungunya, mayaro, and oropouche viruses)
112 made it difficult to determine the real impact of ZIKV on the Brazilian population in
113 the beginning of the outbreak. In fact, the increasing trend of cases of mild fever,
114 pruritus and exanthema in 2014 was hypothesized to be due to dengue, rubella, B19
115 parvovirus, enterovirus, and others arboviruses, especially chikungunya at the time.
116 The association between ZIKV and microcephaly was confirmed only in January of
117 2016 when the virus was detected in fetal nervous system tissue from a Brazilian
118 pregnant woman [10].

119 To face this situation, Brazil created a National Network of specialists in zika
120 and similar diseases (RENEZIKA), a multi-professional team supported by the
121 Ministry of Health with the objectives of: providing research data on surveillance,
122 prevention, control, healthcare and scientific/technological support; contributing to
123 development of guidelines; enhancing epidemiological analysis; fund raising;
124 stimulating participation in scientific events and exchange; and supporting multicenter
125 studies and collaboration on zika infection (available at
126 http://renezika.org/portal/institucional/a-renezika).

127 In addition, the São Paulo Research Foundation (FAPESP) started financing
128 zika research projects in 2016 with incentives to ongoing virology projects in a fast-

track system, currently funding studies to understand the disease and accelerate diagnostics and therapeutics. The work of such team contributed to understanding the burden of the infection and bringing awareness towards the facts versus false assumptions when considering the epidemics. As an example, concerns regarding a possible effect of vaccination or even toxic agents as causes of the observed microcephaly were disseminated in social media and prompted an official clarification by experts. In fact, after efforts by the Brazilian and international scientific community, numerous of ZIKV papers were published, including those focused on the pathogenetic mechanisms associated with central nervous system (CNS) damage by ZIKV using both *in vitro* and *in vivo* models of viral infection.

3. Classification, Viral Structure and Transmission

ZIKV belongs to the *Flavivirus* genus that includes other important human pathogens such as dengue (DENV), west Nile (WNV), yellow fever (YFV) and japanese encephalitis (JEV) viruses, all of them transmitted mainly or exclusively by mosquitoes. *Flavivirus* belongs to the *Flaviviridae* family, which comprises 53 different species [1]. All flaviviruses have a positive-sense, single-stranded RNA genome of around 11 kb containing just one open reading frame (ORF), 5' and 3' untranslated regions (UTRs), and a 5' cap. The ORF encodes a large polyprotein that is cleaved into three structural (C, prM, and E) and eight nonstructural (NS1, NS2A, NS2B, NS3, NS4A, 2K, NS4B, and NS5) proteins [11]. In addition, recent studies have demonstrated the presence of subgenomic RNAs (sfRNAs) during flavivirus replication, which are generated by cleavage by eukaryotic exoribonucleases, repressing of translation through base pairing with cellular mRNAs.

153 Comparative sequence analysis of pre-epidemic and epidemic ZIKV isolates
154 showed some mutations possibly associated with neuropathogenesis [9]. Previous
155 studies in cell cultures, animal models and brain organoids had already shown that
156 ZIKV belonging to the Asian group has a greater ability to infect neural progenitor cells
157 than African-ZIKV (see Pathogenesis section below). Recently, Yuan and colleagues
158 demonstrated that a single mutation very common in ZIKV-isolates from America
159 [7,12], substantially increased ZIKV infectivity in both human and mouse neural
160 progenitor cells. This mutation results in a substitution from a serine to asparagine
161 (S139N) in the viral polyprotein, the 17th amino acid residue of prM protein in a portion
162 prominently exposed at the top of the spikes in immature virions. This location suggests
163 that immature virus particles with specific envelope symmetries could impact cell
164 tropism, pathogenesis and also the presence of alternative routes of viral transmission.
165 Thus, genomic mutations in ZIKV isolates may be behind the explosive dissemination
166 and the high incidence of microcephaly and other neurological symptoms in specific
167 geographic areas, such as the Northeast of Brazil.

168 Genomic mutations can also contribute to the several mechanisms associated
169 with ZIKV transmission in Brazil. The main route of ZIKV transmission is by a
170 mosquito bite (*Aedes sp* and possibly *Culex sp* – discussed in Box 1), with possible
171 vertical transmission by intrauterine mother-to-child route. Interestingly, ZIKV strains
172 isolated from mosquitoes in Brazil do not have the S139N mutation [13], indicating
173 that highly virulent strains could have been disseminated in Brazil by other routes of
174 transmission including sexual transmission. The association between sexual
175 transmission and zika's congenital syndrome needs to be better clarified.

176 The first report of probable non-vector transmission of ZIKV was described in
177 2011, when a patient was infected by ZIKV in Africa and transmitted the virus to his
178 wife in Colorado State, USA, where *Aedes aegypti* is not usually present. After the
179 2015-2016 ZIKV-outbreak, many cases of possible ZIKV sexual transmission were
180 reported (systematically reviewed by Moreira and colleagues [14]), showing that ZIKV
181 can be transmitted from male or female (symptomatic or not) infected individuals by
182 unprotected vaginal, oral or anal intercourse. The hypothesis of sexual transmission
183 was supported by the long-term isolation of infectious ZIKV from semen and female
184 genital tract weeks or months after symptoms onset [14].
185 Sexual transmission of ZIKV is not well understood, but recent findings suggest
186 that the virus primarily infect sperm cells probably via the Tyro3 receptor, which can
187 be found in the midpiece of mature spermatozoa [15]. The same receptor may also be
188 involved in prostate cells infection, and as it is secreted in semen would lead to sexual
189 transmission [16]. Both mechanisms could also occur together, but the first one
190 probably explains better why the virus persists being secreted for months after
191 symptoms onset. In addition, Tyro3 is prominently expressed in neurons, so it is
192 possible that this receptor plays a role in neuropathogenesis of ZIKV [15].
193 Other body fluids have also been reported as possible forms of direct
194 transmission of ZIKV, including breast milk, saliva and urine. However, the role of the
195 ZIKV-transmission by direct contact in Brazil needs further research [17]. In addition,
196 there is evidence of ZIKV transmission by blood transfusion. The contamination of
197 blood banks with ZIKV by asymptomatic donors resulted in at least four reported cases
198 of ZIKV transfusion-transmission in Brazil [18].
199

BOX: Is the *Culex* mosquito a ZIKV vector?

Upon introduction to a new region, arboviruses exposed to different species of possible vectors. The selection of viral strains in different vectors can impact the disease caused by these viruses in humans, affecting pathogenesis and the speed of viral spreading [19]. Thus, transmission by unpredicted vectors could explain some peculiarities of the 2015-2016 zika-Brazilian outbreak, such as neuropathogenesis and the fast spreading. To incriminate an arthropod as a vector, though, some criteria must be followed, including: (i) demonstration of effective contact with the pathogen's host (i. e. feeding); (ii) association in time and space of the vector and the infected host (iii) recurrent demonstration of natural infection of the vector and (iv) experimental transmission of the virus by the vector [20]. Two studies support *Culex* mosquitoes as vectors for ZIKV [20,21] although these studies did not confirm all the criteria [20,22]. In contrast, at least twelve other reports did not agree with this hypothesis (reviewed by [22]). Recently, phylogenetic analyses demonstrated that ZIKV is closely related to WNV and SLEV, which are "Culex-associated" viruses and known to cause neurotrophic associated diseases (reviewed by [23]). Thus, although these conflicting reports may indicate that more than one vector were involved in ZIKV transmission, more studies will be necessary to prove the role of Culex mosquito as vector of ZIKV during Brazilian 2015-2016 outbreak.

4. Clinical presentation

A challenge in addressing ZIKV is its clinical presentation, which can be very mild or even asymptomatic in the majority of infected cases (around 80%), with an incubation period of 4-10 days and duration of disease of 5 to 7 days. Among symptomatic cases, the most frequent conditions are exanthema (maculopapular pruritic rash), fever (low-grade), arthralgia (mostly small joints of hands and feet), non-purulent conjunctivitis, myalgia and prostration [24].

Brazil is an endemic country for other arbovirus and in such scenario [25], with frequent occurrence of DENV for example, many of the symptomatic cases do not seek medical care unless there is a severe feature, which might present after the optimal timing for sample collection and diagnosis.

Guillain-Barre Syndrome (GBS) was first described in association with zika in the French Polynesian outbreak of 2013-2014 [2]. GBS is defined as an acute paralysis, with areflexic response, with noted high levels of protein in the cerebrospinal fluid and normal cell counts (albuminocytologic dissociation). It is the most prevalent cause of

233 flaccid paralysis and up to 20% of affected patients persist with disabilities; around 5%
234 die due to the complication [26]. Usually GBS presents as a progressive, bilateral and
235 symmetric limb weakness of immune-mediated response. In the last couple of years,
236 ZIKV-associated GBS has been reported in different settings, including Brazil,
237 Colombia, the United States, Martinique and others [27].

238 A broad range of complications may present associated with maternal ZIKV
239 infection, including fetal and newborn neurological and ocular anomalies, fetal growth
240 restriction (FGR), stillbirth, and perinatal death. *Congenital zika Syndrome* is the
241 designated term to refer to these cases, with microcephaly as one of the clinical signs
242 reported, including pronounced brain damage, mainly characterized by reduced cortical
243 development and atrophy, arthrogryposis and hypoplasia of the cerebellum and
244 cerebellar vermis. Polyhydramnios is a common finding, probably in response to
245 swallowing impairment due to brain injury [28,29].

246 The prevalence of these findings is largely understudied, especially considering
247 limitations in identification and testing of cases. Results from the US zika pregnancy
248 registry collaboration showed that among pregnancies identified with zika virus
249 infection there was an overall 6% of associated birth defects or 11% when infection had
250 occurred during first trimester [28]. However, *congenital zika syndrome* has been
251 reported in cases of infection in the second and even third trimesters [30].

252 The ZIKV precise mechanisms for maternal-fetal transmission are largely
253 unknown. The same is true for the TORCH infections (acronym for *Toxoplasma gondii*,
254 *Treponema pallidum*, rubella virus, cytomegalovirus (CMV) and herpes simplex virus
255 (HSV) [31].

256

5. ZIKV and the placenta

Potential routes of ZIKV vertical transmission include: breaks in the syncytiotrophoblast (SYN) layer, the multinucleated, differentiated cells in direct contact with maternal blood; direct infection of the SYN layer (antibody transcytosis); infection of extravillous trophoblasts (EVTs), the anchoring cells attached to the uterus; or through the decidua, modified cells in the uterine wall for implantation of pregnancy and/or maternal microvasculature. Most likely, vertical transmission is different between the early and later (>12 weeks) stages of human pregnancy [32].

The study of all placentas from confirmed or suspected cases is an official recommendation, as part of standard healthcare for these women and their babies. zika virus RNA testing (via qRT-PCR) on fixed and frozen tissue is very important and might even confirm fetal infection, since serological testing has limitations due to timing of the test, depending on the accurate window of ZIKV detection [33].

Nevertheless, protocols for placental sampling and storage differ worldwide. Although the Brazilian Ministry of Health has an official guideline, it includes no figures to explain systematic placental sampling and only a few words describing a 3 fragments sampling of 1.0x1.0cm pieces, with no specific detailing on depth needed or sites of collection expected (available at http://combateaedes.saude.gov.br/en/). However, not all medical settings have the opportunity for placental pathology analysis, which adds on to the need to adequately transport samples to other facilities.

Placental pathologic findings have been reported as nonspecific and mild, comprising chronic placentitis (TORCH type), increased Hofbauer cells, chronic villitis, villous immaturity, increased vascularity stromal fibrosis and calcification, variable perivilous fibrin and mononuclear cells, and also lymphocytic deciduitis and

281 focal syncytiotrophoblast necrosis. Most of the detailed cases were from first trimester
282 infections and clinical symptomatic cases, which can explain the severity, foremost
283 with significant cases of abortions, stillbirth or neonatal death [34,35].
284
285 **6. Pathogenesis: Cell Tropism and the Effects of ZIKV Infection and Replication**
286
287 Understanding the pathogenesis of ZIKV infection has been the subject of
288 intense research for which the tropism of ZIKV is crucial. After ZIKV delivery by a
289 blood-feeding female mosquito, the infection begins through permissive skin cells.
290 ZIKV infects human primary skin fibroblast, keratinocytes and immature dendritic
291 cells. Receptors involved in ZIKV entry include TIM-1 and AXL, important for
292 permissiveness of human skin fibroblasts to infection and replication of ZIKV. Herein,
293 ZIKV-infected primary fibroblasts displayed autophagosome formation related to
294 increased viral replication. ZIKV target cells such as macrophages, dendritic cells, T
295 cells, immature NKs, sertoli cells, retinal pigment epithelial cells and endothelial cells
296 showed high expression of TYRO3, AXL and MER receptors in their surface. Through
297 them, ZIKV can bind and enter cells by endocytosis. However, these receptors are not
298 crucial for virus entry into T cells or for ZIKV infection in a mouse model [16].
299 As expected, due to the neurological complications reported, an increasing
300 number of studies have shown ZIKV in different types of neural and placental cells.
301 The neurological complications observed in ZIKV infection are closely related to ZIKV
302 preferential infection triggering apoptosis in neural progenitor cells (NPCs) [36,37].
303 The infection by the MR766 ZIKV strain in cell culture was more effective in NPCs
304 compared with mature cortical neurons [37] efficiently infecting neural cell-derived

organoids resulting in a decrease in overall organoid size [36]. The ZIKV infection impact on neural development was demonstrated in a mouse fetus model, where ZIKV inoculation in brain resulted in cell-cycle arrest, apoptosis, and inhibition of NPC differentiation and cortical thinning and microcephaly [38]. Stem cells from the ventricular zone and neurons from corticospinal pyramidal were also targeted by ZIKV [39]. Yet, ZIKV infection in human neural stem cells growing as neurospheres and brain organoids resulted in. reduced growth and viability. In addition, ZIKV infection was demonstrated in cranial neural crest cells leading to high levels ofneurodevelopmental cytokines that can kill or cause aberrant differentiation of neural progenitors [40].

Microcephaly is the most critical neurological complication and is associated with congenital abnormalities. Many studies have addressed how the virus can cross the placenta, alter normal fetal development, and disrupt specific cellular functions. In this context, ZIKV RNA was detected in amniotic fluid samples of pregnant women whose fetuses were diagnosed with microcephaly [41]. Viral RNA and protein were detected in newborn and fetal brain and placental tissues [10,42]. ZIKV is able to infect and replicate in human placental macrophages, called Hofbauer cells, and to a lesser extent in cytotrophoblasts [43].

However, the tropism of ZIKV is more widespread once it has been detected in other tissues and body fluids. Studies on eye damage demonstrated that ZIKV RNA was present in murine ocular tissue such as cornea, retina and optical nerve, and in human ZIKV it was detected in conjunctival fluid. ZIKV also targets cells from the reproductive tract including spermatogonia and Sertoli cells in males, which could be

involved in long term sterility, and vaginal epithelium and uterine fibroblast in females [16] .

6.1 Pathogenesis: Immune Response to ZIKV Infection

Immunocompetent mice are not natural hosts for the virus, in part because the ZIKV NS5 protease is not able to degrade mouse STAT2, a transcription factor activated downstream the IFN receptor signaling, as opposed to human [44]. Therefore, IFNAR1 knockout mice, neonatal wild type animals or direct inoculation of virus in immunocompetent cells have to be used to sustain infection in the murine model. Pathogenesis studies of ZIKV infection in a mouse model have used IFN knockout animals, which presumably bypasses innate immune responses in peripheral organs.

Inoculation of ZIKV into neonatal mice resulted in severe neurological disease associated with infiltration of T cells in the CNS, weight loss, and death in a subset of animals [45]. Adult IFNAR1 knockout mice showed placental infection, placenta-fetal transmission and neurological complications [46]. Infection and death by apoptosis was observed in neural progenitor cells in IRF3, IRF5, and IRF7 knockout mice models [47]. ZIKV has been detected in the blood, spleen, brain, spinal cord, kidney and eye of immunocompromised adult mice.

The pathogenesis underlying the congenital neurological complications observed in humans, such as intrauterine growth restriction, microcephaly, and miscarriage [48], also has been extensively studied in mice. As reviewed previously [16], it was shown in type I interferon (IFN)-deficient mouse models of ZIKV transmission in utero that ZIKV infects different trophoblasts and fetal endothelial cells of the placenta. Subsequently it was shown that the virus can cross the placenta to infect

352 the fetal head, with the placenta and fetus more susceptible to ZIKV infection at earlier
353 gestational stages. This mouse model also revealed intrauterine growth restriction and
354 fetal demise. Infection of pregnant Ifnar1$^{-/-}$ mice with a Brazilian ZIKV strain resulted
355 in intrauterine growth restriction and reduced fetus size without demise. When the same
356 strain was used at high doses in wild-type pregnant mice, fetuses showed intrauterine
357 growth restriction and microcephaly.

358 In the context of maternal-fetal interface and antiviral defense, it was
359 demonstrated that ZIKV replication in Houfbaer cells, trophoblasts and neuroblasts is
360 dependent on interferon signaling by JAK-STAT. Inhibition of this pathway resulted in
361 increased viral replication [49]. A recent study used 3D cell-line-based models of
362 human syncytiotrophoblasts and showed that constitutive release of type III IFNs
363 (IFNλ1 and IFNλ2) induced resistance to ZIKV infection [50]. Autophagy also plays
364 a major role in ZIKV infection as revealed by studying pregnant Atg16l1-deficient mice
365 or through autophagy chemical inhibition in pregnant ZIKV-infected mice [51]. This
366 study demonstrated that autophagy inhibition is favorable for the host to limit vertical
367 transmission. Moreover, the authors discuss the possible shift from degradative
368 autophagy to secretory autophagy and its relevance on release and spreading of mature
369 viral particles. Endothelial cells might also be crucial in ZIKV vertical transmission.

370 To cross an endothelial barrier such as the blood brain barrier (BBB), ZIKV
371 disrupts the tight junctions and travels between the cells or infects the endothelial cells
372 directly, consequently causing cell lysis and releasing virus particles across the
373 membrane. Indeed, Liu et al. demonstrated that ZIKV can infect immortalized human
374 cerebral microvessel endothelial cells (hCMEC/D3) [52]. Moreover, Tabata et al.
375 demonstrated that many cell types, including human umbilical vein endothelial cells

(HUVECs) which comprises the placenta barrier, are also susceptible to ZIKV infection [53]. Murine models have demonstrated loss and damage of blood vessels in the placenta, indicating that endothelial cells play a crucial role in vertical transmission of ZIKV [54].

Recently, primary human brain microvascular ECs (hBMECs) have been considered as ZIKV reservoirs [55]. ZIKV replicates persistently in hBMECs with little or no cytopathology through ZIKV-directed cell survival responses. In fact, ZIKV does not permeabilize hBMECs and is released basolaterally from polarized hBMECs, suggesting a direct mechanism for ZIKV to cross the BBB. Interestingly, CCL5 was identified as one of the regulated immune response mediators under ZIKV infection and the authors explore its role in trafficking immune cells to the BBB, and both foster ZIKV's spread to neurons and inflammatory CNS pathology [55].

In addition to direct infection or compromising tight junctions, the virus may be transported through the CNS by infected leukocytes; a mechanism called the "Trojan Horse" pathway [56]. This possible route could explain how ZIKV reaches immune-privileged sites and breaks endothelial barriers such as the placental-fetal and brain-blood. In this regard, two recent studies demonstrated that among the peripheral blood white cells, monocytes are the dominant cell type infected by ZIKV infection. This was observed in infections mediated by both Asian and African strains. The infection also promotes an increase in monocytes number, especially for CD16+ non-classical and intermediate subtypes (characterized by inflammatory role or both phagocytic and inflammatory functions) [57,58]. Additionally, Foo and colleagues identified high levels of IL-10 in the plasma of pregnant women infected with the ZIKV Asian strain, suggesting that ZIKV could be related to immunosuppression.

Leukocyte adhesion to brain microvascular endothelial cells is commonly mediated by several surface proteins including ICAM-1 and E-selectin [59]. Other flaviviruses such as WNV have been shown to increase expression of these surface proteins *in vivo* and *in vitro* [59]. Indeed, host cells secrete cytokines to recruit a rapid immune response to inactivate viral expression following infection, which is an important part of the innate host immune response to viruses. Therefore, considering host immune modulation during ZIKV infection, we recently analyzed 21 immune mediators by microbead-based immunoassays [60]. We observed that immune makers were highly interconnected and were involved in the NF-κB signaling pathway. Moreover, we detected MCP-1 (CCL2) and SDF-1 (CXCL12) in patient sera, chemokines already described as involved in leukocyte transmigration across the BBB in viral infections [56] (Fig. 2).

6.2 Cross-reactive immunity amongst flaviviruses

ZIKV-DENV ADE: Antibody-dependent enhancement (ADE) is a well-known process on DENV infection. DENV is also a Flavivirus, with four serotypes. The ADE phenomenon is related to the disease severity increase in secondary infection. This occurs because non-neutralizing antibodies bind to Fc receptors bearing cells (such as macrophages), leading to higher viremia and production of high levels of inflammatory cytokines [61]. Antibodies to the structural precursor-membrane protein (prM) and E protein of DENV and the consequent infectivity of immature viral particles are a major components of dengue ADE [62]. Although ZIKV appears to exist as a single serotype, individuals in endemic areas have been reported to experience sequential infections

423 with both viruses [63]. ZIKV and DENV are closely related and share high amino acid
424 identity, thus supporting a possibly similar cross-reactivity between ZIKV and DENV.
425 Exploring memory lymphocytes on ZIKV-infected mice, Stettler and
426 colleagues [63] found monoclonal antibodies against the E protein (domain I/II) that
427 cross-reacted and effectively enhanced ZIKV and DENV infections *in vitro* as well as
428 in DENV-infected mice. Interestingly, transfer of DENV convalescent human plasma
429 enhanced pathogenesis in a mouse model of ZIKV infection [64]. Moreover, an anti-
430 DENV E protein monoclonal antibody cross-reacted with ZIKV, neutralized it and
431 greatly enhanced the replication of viral RNA. DENV-immune plasma and monoclonal
432 antibodies to DENV potently enhanced ZIKV infection, suggesting that preexisting
433 immunity to DENV might increase ZIKV replication and disease severity [65]. In
434 contrast, studies with patients did not support ADE between dengue and zika, since nor
435 viremia neither cytokines levels were higher in ZIKV patients with prior DENV [66].
436 Thus, differences between ZIKV and DENV tropism, human genetic background and
437 geographic behaviors could impact the clinical outcome after subsequent flavivirus
438 infections.
439 ZIKV, DENV and YFV: CD8+ T cells in common: CD8+ T cells mediate immunity to
440 intracellular infections by recognizing and responding to specific peptides that are
441 derived from intracellular proteins and presented on class I major histocompatibility
442 complex (MHC) molecules. These cells are important for clearance of virus-infected
443 cells and relatively little is known about cross-reactive CD8+ T cell-mediated immunity
444 amongst flaviviruses [63,64]. Upon ZIKV infection, the mouse model lacking the type
445 I interferon receptor showed that the virus replicated and induced a robust CD8+ T cell
446 response [67]. Furthermore, CD8+ T cells were shown to reduce viral replication and

attenuate disease. Using HLA transgenic mouse model, zika virus elicited antigen-specific CD8+ T cell responses and DENV-immune mice challenged with ZIKV produced ZIKV/DENV cross-reactive epitopes able to elicit a CD8+ T cell responses that reduced infectious ZIKV levels [68].

During the Brazilian outbreak an increased microcephaly occurrence was reported in regions with lower yellow fever vaccination coverage [69]. The last epidemiological data published by the Brazilian Ministry of Health showed that in some federative units with low vaccination coverage the number of confirmed Congenital zika virus syndrome cases was higher, but we were not able to establish any statistical correlation. This result, therefore, should be widely and carefully revisited using up-to-date epidemiological data on both vaccination coverage and the prevalence of congenital zika syndrome. Recent findings indicate that the YFV vaccine generates CD8+ T cell-mediated immune responses against zika virus [70]. Thus, more studies will be necessary in order to determine the role of an anti-YFV immunological response during ZIKV infection.

7. Conclusions and Future Perspectives

The number of studies on ZIKV has greatly increased in the last 2 years since the epidemics in Brazil was acknowledged, involving both national and international collaboration. Research on animal models and infected human samples has helped to understand the pathology of the disease and epidemiology reports have enabled the understanding of virus dissemination. In the last two years, ZIKV was proved as a cause of congenital malformations and ophthalmological and neurological dysfunctions in adults and children. Although the circulation of this virus has decreased substantially

in Brazil during 2017, the maintenance of this virus in vertebrate and arthropod reservoirs in nature indicate that new ZIKV epidemics can happen in the near future. Thus, surveillance programs for detection of ZIKV and others arbovirus in humans and other animal reservoirs need to be maintained and encouraged in tropical countries such as Brazil. Moreover, many unanswered questions need to be clarified, especially those regarding why there are so many cases of severe disease in certain regions of the Americas, such as Northeastern Brazil. The characterization of possible risk factors (such as human genetic background and microbiome directly affected by socioeconomic conditions), the impact of co-infections, possible new transmission routes, determinants of placental infection and the mechanisms of host control and immune evasion (e.g., how does the virus cross the endothelial barrier) are research gaps that need to be addressed in the next years.

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Potential conflicts of interest.

All authors do not have conflicts of interest.

References

[1] Musso D, Gubler DJ. Zika virus. Clin Microbiol Rev 2016;29:487–524.

[2] Cao-Lormeau VM, Blake A, Mons S, Lastere S, Roche C, Vanhomwegen J, et al. Guillain-Barré Syndrome outbreak associated with Zika virus infection in French Polynesia: a case-control study. Lancet (London, England) 2016;387:1531–9.

[3] PAHO, World Health Organization (WHO). Zika-Epidemiological Report - Brazil. 2017. (http://www.paho.org/hq/index.php?option=com_docman&task=doc_view&gid=35221&&Itemid=270&lang=en).

[4] Victora CG, Barreto ML, do Carmo Leal M, Monteiro CA, Schmidt MI, Paim J, et al. Health conditions and health-policy innovations in Brazil: the way forward. Lancet 2011;377:2042–53.

[5] Guimarães R, Santos LMP, Angulo-Tuesta A, Serruya SJ. Defining and implementing a national policy for science, technology, and innovation in health: lessons from the Brazilian experience. Cad Saude Publica 2006;22:1775–85.

[6] Assis AMO, Barreto ML, Santos NS, Oliveira LPM de, Santos SMC dos, Pinheiro SMC. Desigualdade, pobreza e condições de saúde e nutrição na infância no Nordeste brasileiro. Cad Saude Publica 2007;23:2337–50.

[7] Faria NR, Azevedo R d. S d. S, Kraemer MUG, Souza R, Cunha MS, Hill SC, et al. Zika virus in the Americas: Early epidemiological and genetic findings. Science 2016;352:345–9.

[8] Passos SRL, Borges dos Santos MA, Cerbino-Neto J, Buonora SN, Souza TML, de Oliveira RVC, et al. Detection of zika virus in April 2013 patient samples, Rio de Janeiro, Brazil. Emerg Infect Dis 2017;23:2120–1.

[9] Faria NR, Quick J, Claro IM, Thézé J, de Jesus JG, Giovanetti M, et al. Establishment and cryptic transmission of Zika virus in Brazil and the Americas. Nature 2017;546:406–10.

[10] Mlakar J, Korva M, Tul N, Popović M, Poljšak-Prijatelj M, Mraz J, et al. Zika virus associated with microcephaly. N Engl J Med 2016;374:951–8.

[11] Pierson TC, Diamond MS Flaviviruses, in Knipe DM, Howley PM (Eds). Fields Virology, 6th ed, Wolters Kluwer/Lippencott Williams and Wilkins, Philadelphia, PA, USA, 2013 pp. 747-94.

[12] Yuan L, Huang X-Y, Liu Z-Y, Zhang F, Zhu X-L, Yu J-Y, et al. A single mutation in the prM protein of Zika virus contributes to fetal microcephaly. Science 2017; 358(6365):933-936.

[13] Wang L, Valderramos SG, Wu A, Ouyang S, Li C, Brasil P, et al. From Mosquitos to Humans: Genetic Evolution of Zika Virus. Cell Host Microbe 2016;19:561–5.

[14] Moreira J, Peixoto TM, Siqueira AM, Lamas CC. Sexually acquired Zika virus: a systematic review. Clin Microbiol Infect 2017;23:296–305.

[15] Bagasra O, Addanki KC, Goodwin GR, Hughes BW, Pandey P, McLean E. Cellular Targets and Receptor of Sexual Transmission of Zika Virus. Appl Immunohistochem Mol Morphol. 2017; 25(10):679-68.

[16] Miner JJ, Diamond MS. Zika Virus Pathogenesis and Tissue Tropism. Cell Host Microbe 2017;21:134–42.

[17] Aliota MT, Bassit L, Bradrick SS, Cox B, Garcia-Blanco MA, Gavegnano C, et al. Zika in the Americas, year 2: What have we learned? What gaps remain? A report from the Global Virus Network. Antiviral Res 2017;144:223–46.

[18] Slavov SN, Hespanhol MR, Rodrigues ES, Levi JE, Ubiali EMA, Covas DT, et al. Zika virus RNA detection in asymptomatic blood donors during an outbreak in the northeast region of São Paulo State, Brazil, 2016. Transfusion 2017. doi:10.1111/trf.14322. "Epub ahead of print"

[19] Marcondes CB, Contigiani M, Gleiser RM, Reisen W. Emergent and reemergent arboviruses in South America and the Caribbean: Why so many and why now? J Med Entomol 2017;54:509–32.

[20] Guedes DR, Paiva MH, Donato MM, Barbosa PP, Krokovsky L, Rocha SW dos S, et al. Zika virus replication in the mosquito *Culex quinquefasciatus* in Brazil. Emerg Microbes Infect 2017;6:e69.

[21] Guo X, Li C, Deng Y, Xing D, Liu Q, Wu Q, et al. *Culex pipiens quinquefasciatus*: a potential vector to transmit Zika virus. Emerg Microbes Infect 2016;5:e102.

[22] Van den Hurk AF, Hall-Mendelin S, Jansen CC, Higgs S. Zika virus and *Culex quinquefasciatus* mosquitoes: a tenuous link. Lancet Infect Dis 2017; 17:1014–6.

[23] Hunter FF. Linking only *Aedes aegypti* with zika virus has world-wide public health implications. Front Microbiol 2017;8: 1248.

564 [24] Baud D, Gubler DJ, Schaub B, Lanteri MC, Musso D. An update on Zika virus
565 infection. Lancet 2017; 390(10107):2099-2109.

566 [25] Figueiredo LTM. The recent arbovirus disease epidemic in Brazil. Rev Soc
567 Bras Med Trop 2015;48:233–4.

568 [26] Yuki N, Hartung H-P. Guillain–Barré Syndrome. N Engl J Med
569 2012;366:2294–304.

570 [27] Nascimento OJM, da Silva IRF. Guillain–Barré syndrome and Zika virus
571 outbreaks. Curr Opin Neurol 2017;30:500–7.

572 [28] Honein MA, Dawson AL, Petersen EE, Jones AM, Lee EH, Yazdy MM, et al.
573 Birth Defects Among Fetuses and Infants of US Women With Evidence of
574 Possible Zika Virus Infection During Pregnancy. JAMA 2017;317:59–68.

575 [29] Chimelli L, Melo ASO, Avvad-Portari E, Wiley CA, Camacho AHS, Lopes VS, et
576 al. The spectrum of neuropathological changes associated with congenital
577 Zika virus infection. Acta Neuropathol 2017;133:983–99.

578 [30] França GVA, Schuler-Faccini L, Oliveira WK, Henriques CMP, Carmo EH, Pedi
579 VD, et al. Congenital Zika virus syndrome in Brazil: a case series of the first
580 1501 livebirths with complete investigation. Lancet 2016; 388
581 (10047):891-7.

582 [31] Neu N, Duchon J, Zachariah P. TORCH Infections. Clin Perinatol 2015;42:77–
583 103.

584 [32] Adibi JJ, Marques ETA, Cartus A, Beigi RH. Teratogenic effects of the Zika
585 virus and the role of the placenta. Lancet 2016;387:1587–90.

586 [33] CDC. Zika virus: collection and submission of specimens for zika virus
587 testing at time of birth 2017. (https://www.cdc.gov/zika/pdfs/collection-
588 submission-specimens-zika-testing-at-birth.pdf).

589 [34] Ritter JM, Martines RB, Zaki SR. Zika Virus: Pathology from the pandemic.
590 Arch Pathol Lab Med 2017;141:49–59.

591 [35] Noronha L de, Zanluca C, Azevedo MLV, Luz KG, Santos CND Dos. Zika virus
592 damages the human placental barrier and presents marked fetal
593 neurotropism. Mem Inst Oswaldo Cruz 2016;111:287–93.

594 [36] Dang J, Tiwari SK, Lichinchi G, Qin Y, Patil VS, Eroshkin AM, et al. Zika virus
595 depletes neural progenitors in human cerebral organoids through
596 activation of the innate immune receptor TLR3. Cell Stem Cell 2016;19:258–
597 65.

598 [37] Tang H, Hammack C, Ogden SC, Wen Z, Qian X, Li Y, et al. Zika virus infects
599 human cortical neural progenitors and attenuates their growth. Cell Stem
600 Cell 2016;18:587–90.

601 [38] Li C, Xu D, Ye Q, Hong S, Jiang Y, Liu X, et al. Zika virus disrupts neural
602 progenitor development and leads to microcephaly in mice. Cell Stem Cell
603 2016;19:120–6.

604 [39] Garcez PP, Loiola EC, Madeiro da Costa R, Higa LM, Trindade P, Delvecchio
605 R, et al. Zika virus impairs growth in human neurospheres and brain
606 organoids. Science 2016;352:816–8.

607 [40] Bayless NL, Greenberg RS, Swigut T, Wysocka J, Blish CA. Zika virus infection
608 induces cranial neural crest cells to produce cytokines at levels detrimental
609 for neurogenesis. Cell Host Microbe 2016;20:423–8.

610 [41] De Araújo TVB, Rodrigues LC, de Alencar Ximenes RA, de Barros Miranda-
611 Filho D, Montarroyos UR, de Melo APL, et al. Association between Zika virus
612 infection and microcephaly in Brazil, January to May, 2016: preliminary
613 report of a case-control study. Lancet Infect Dis 2016;16:1356–63.

614 [42] Martines RB, Bhatnagar J, Keating MK, Silva-Flannery L, Muehlenbachs A,
615 Gary J, et al. Notes from the Field : Evidence of zika virus infection in brain
616 and placental tissues from two congenitally infected newborns and two
617 fetal losses — Brazil. MMWR Morb Mortal Wkly Rep 2016; 19;65(6):159-
618 60.

619 [43] Quicke KM, Bowen JR, Johnson EL, McDonald CE, Ma H, O'Neal JT, et al. Zika
620 virus infects human placental macrophages. Cell Host Microbe 2016;20:83–
621 90.

622 [44] Kumar A, Hou S, Airo AM, Limonta D, Mancinelli V, Branton W, et al. Zika
623 virus inhibits type-I interferon production and downstream signaling.
624 EMBO Rep 2016;17:1766–75.

625 [45] Lazear HM, Govero J, Smith AM, Platt DJ, Miner JJ, Diamond MS. A mouse
626 model of zika virus pathogenesis. Cell Host Microbe 2016;19(5):720-30.

627 [46] Rossi SL, Tesh RB, Azar SR, Muruato AE, Hanley KA, Auguste AJ, et al.
628 Characterization of a novel murine model to study zika virus. Am J Trop Med
629 Hyg 2016;94:1362–9.

630 [47] Li H, Saucedo-Cuevas L, Regla-Nava JA, Chai G, Sheets N, Tang W, et al. Zika
631 virus infects neural progenitors in the adult mouse brain and alters
632 proliferation. Cell Stem Cell 2016;19:593–8.

633 [48] Brasil P, Pereira JP, Moreira ME, Ribeiro Nogueira RM, Damasceno L,
634 Wakimoto M, et al. Zika virus infection in pregnant women in Rio de Janeiro.
635 N Engl J Med 2016; 375(24):2321-2334

636 [49] Gavegnano C, Bassit LC, Cox BD, Hsiao H-M, Johnson EL, Suthar M, et al. Jak
637 inhibitors modulate production of replication competent zika virus in
638 human hofbauer, trophoblasts, and neuroblastoma cells. Pathog Immun
639 2017;2:199-218.

640 [50] Corry J, Arora N, Good CA, Sadovsky Y, Coyne CB. Organotypic models of
641 type III interferon-mediated protection from Zika virus infections at the
642 maternal–fetal interface. Proc Natl Acad Sci 2017; 114(35):9433-9438.

643 [51] Cao B, Parnell LA, Diamond MS, Mysorekar IU. Inhibition of autophagy limits
644 vertical transmission of Zika virus in pregnant mice. J Exp Med
645 2017;214:2303–13.

646 [52] Liu S, Delalio LJ, Isakson BE, Wang TT. AXL-mediated productive infection
647 of human endothelial cells by zika virus. Circ Res 2016;119:1183–9.

648 [53] Tabata T, Petitt M, Puerta-Guardo H, Michlmayr D, Wang C, Fang-Hoover J,
649 et al. Zika virus targets different primary human placental cells, suggesting
650 two routes for vertical transmission. Cell Host Microbe 2016;20:155–66.

651 [54] Miner JJ, Cao B, Govero J, Smith AM, Fernandez E, Cabrera OH, et al. Zika
652 virus infection during pregnancy in mice causes placental damage and fetal
653 demise. Cell 2016;165:1081–91.

654 [55] Mladinich MC, Schwedes J, Mackow ER. Zika virus persistently infects and is
655 basolaterally released from primary human brain microvascular
656 endothelial cells. MBio 2017;8:e00952–17.

657 [56] Miner JJ, Diamond MS. Mechanisms of restriction of viral neuroinvasion at
658 the blood–brain barrier. Curr Opin Immunol 2016;38:18–23.

659 [57] Foo S-S, Chen W, Chan Y, Bowman JW, Chang L-C, Choi Y, et al. Asian Zika
660 virus strains target CD14+ blood monocytes and induce M2-skewed
661 immunosuppression during pregnancy. Nat Microbiol 2017; 2(11):1558-
662 1570.

663 [58] Michlmayr D, Andrade P, Gonzalez K, Balmaseda A, Harris E. CD14+CD16+
664 monocytes are the main target of Zika virus infection in peripheral blood
665 mononuclear cells in a paediatric study in Nicaragua. Nat Microbiol 2017;
666 2(11):1462-1470.

667 [59] Roe K, Orillo B, Verma S. West nile virus-induced cell adhesion molecules on
668 human brain microvascular endothelial cells regulate leukocyte adhesion
669 and modulate permeability of the in vitro blood-brain barrier model. PLoS
670 One 2014; 9(7):e102598.

671 [60] Kam Y-W, Leite JA, Lum F-M, Tan JJL, Lee B, Judice CC, et al. Specific
672 biomarkers associated with neurological complications and congenital
673 central nervous system abnormalities from zika virus–infected patients in
674 Brazil. J Infect Dis 2017;216(2):172-181.

675 [61] Screaton G, Mongkolsapaya J, Yacoub S, Roberts C. New insights into the
676 immunopathology and control of Dengue virus infection. Nat Rev Immunol
677 2015;15:745–59.

678 [62] Da Silva Voorham JM, Rodenhuis-Zybert IA, Nuñez NVA, Colpitts TM, van
679 der Ende-Metselaar H, Fikrig E, et al. Antibodies against the envelope
680 glycoprotein promote infectivity of immature dengue virus serotype 2. PLoS
681 One 2012;7(3):e29957.

682 [63] Stettler K, Beltramello M, Espinosa D a., Graham V, Cassotta a., Bianchi S, et
683 al. Specificity, cross-reactivity, and function of antibodies elicited by Zika
684 virus infection. Science 2016;353:823–6.

685 [64] Bardina S V., Bunduc P, Tripathi S, Duehr J, Frere JJ, Brown J a., et al.
686 Enhancement of Zika virus pathogenesis by preexisting antiflavivirus
687 immunity. Science 2017;356:175–80.

688 [65] Dejnirattisai W, Supasa P, Wongwiwat W, Rouvinski A, Barba-spaeth G,
689 Duangchinda T, et al. Dengue virus sero-cross-reactivity drives antibody-
690 dependent enhancement of infection with zika virus. Nat Immunol 2016; 7:
691 1102–1108.

692 [66] Terzian ACB, Schanoski AS, Mota MT de O, da Silva RA, Estofolete CF,
693 Colombo TE, et al. Viral load and cytokine response profile does not support
694 antibody-dependent enhancement in dengue-primed zika virus–infected
695 patients. Clin Infect Dis 2017; 65(8):1260-1265.

696 [67] Elong Ngono A, Vizcarra EA, Tang WW, Sheets N, Joo Y, Kim K, et al. Mapping
697 and role of the cd8(+) t cell response during primary zika virus infection in
698 mice. Cell Host Microbe 2017;21:35–46.

699 [68] Wen J, Tang WW, Sheets N, Ellison J, Sette A, Kim K, et al. Identification of
700 Zika virus epitopes reveals immunodominant and protective roles for
701 dengue virus cross-reactive CD8+ T cells. Nat Microbiol 2017;2:17036.

[69] De Góes Cavalcanti LP, Tauil PL, Alencar CH, Oliveira W, Teixeira MM, Heukelbach J. Zika virus infection, associated microcephaly, and low yellow fever vaccination coverage in Brazil: is there any causal link? J Infect Dev Ctries 2016;10:563.

[70] Blom K, Sandberg JT, Loré K, Ljunggren H-G. Prospects for induction of CD8 T cell-mediated immunity to Zika virus infection by yellow fever virus vaccination. J Intern Med 2017;282:206–8.

Figures

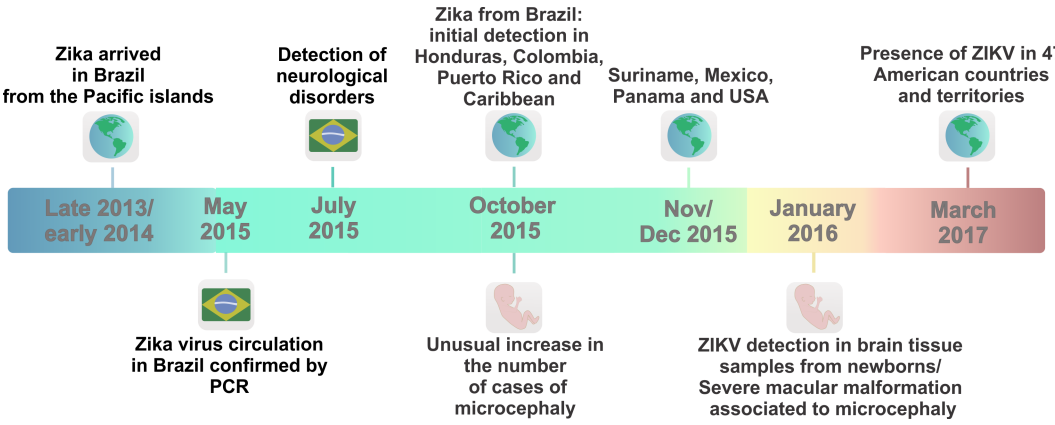

Figure 1. Timeline of Zika virus outbreak in Brazil and subsequent spread in the Americas.

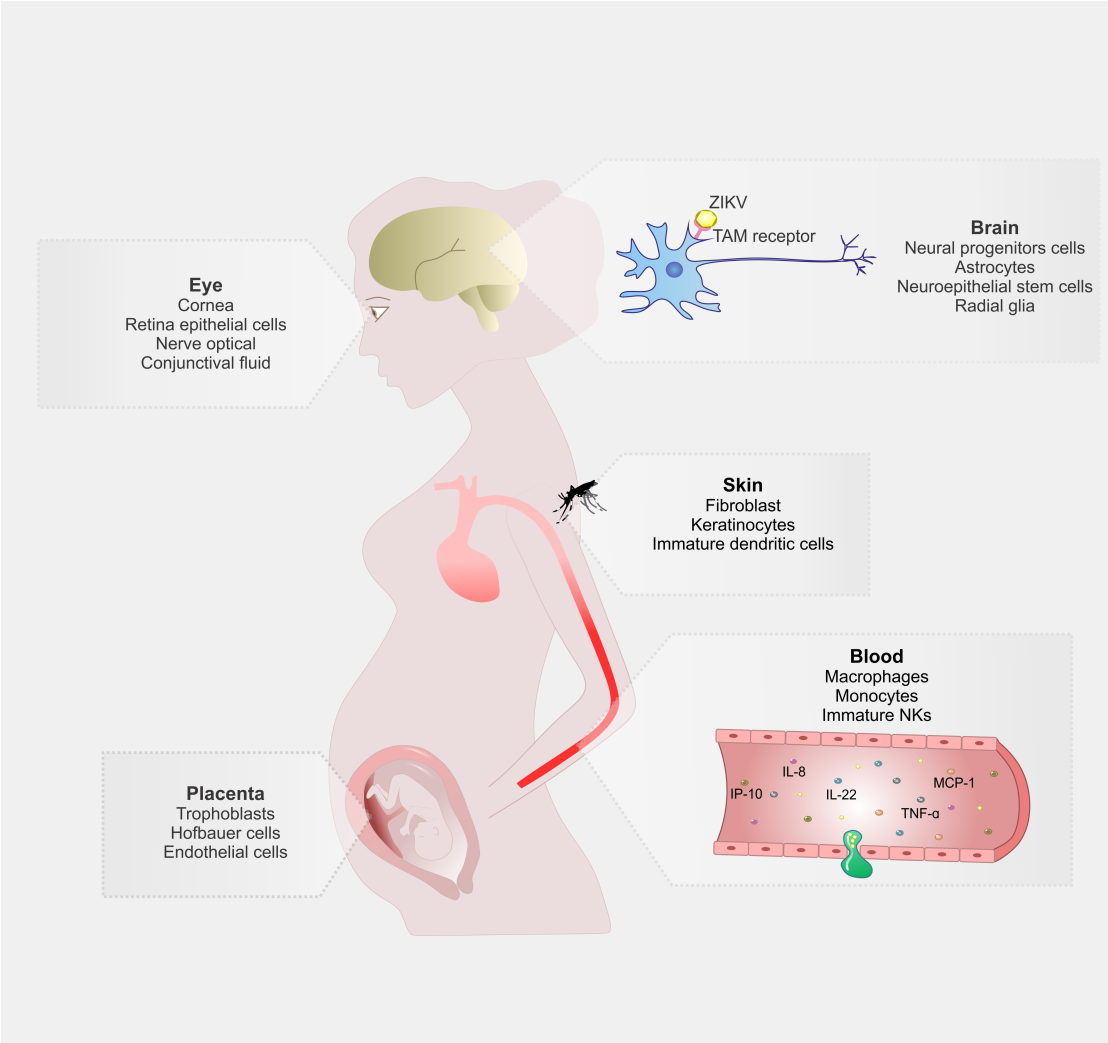

Figure 2. Zika virus cell tropism and pathogenesis. ZIKV targets many different cell types; most of them display TAM receptors on their surface. The neurological complications are essentially due to ZIKV infection of neural cells (neural progenitor cells, astrocytes, radial glia and neuroepithelial stem cells) and placental cells (trophoblasts, Hofbauer cells and endothelial cells). ZIKV also targets cells from other body sites including skin (fibroblasts, keratocytes and immature dendritic cells), eye (cornea and retina epithelial cells, nerve optical and also fluids) and blood (macrophages, monocytes and immature natural killers- NKs). ZIKV infection progression can be observed by circulation of specific cytokines (e.g. IP-10, MCP-1, IL-8, IL-22 and TNF-α).