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REVIEW

Zika virus in the eye of the cytokine storm

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ABSTRACT. Zika virus (ZIKV) is an emerging arbovirus that causes a mosquito-borne disease. Although infection with ZIKV generally leads to mild disease, its recent emergence in the Americas has been associated with an increase in the development of the Guillain-Barré syndrome in adults, as well as with neurological complications, in particular congenital microcephaly, in new-borns. Over the five past years, through the combined efforts of the scientific community, comprehensive remarkable progress aimed at deciphering the clinical, virological, physiopathological, and immunological features of ZIKV infection. This review highlights some of the most recent advances in our understanding of the role of cytokines and chemokines in ZIKV infection, and discusses potential links to pathogenesis.

Key words: Zika virus, Flavivirus, Cytokines, Chemokines, Microcephaly, Guillain-Barré syndrome

INTRODUCTION

Zika virus (ZIKV) is an emerging arbovirus of the *flavivirus* genus discovered in 1947 near Entebbe, Uganda, where it circulates in the forests between nonhuman primates and sylvatic mosquitoes [1]. Until 2007, ZIKV has silently circulated in many parts of Africa and Asia, with less than 20 documented human infections that represented only cases of spillover transmission from the sylvatic cycle, in which humans became infected as an accidental host [2]. The first large outbreak of human infection by ZIKV occurs in 2007 in Micronesia [3], followed by the one in French Polynesia in late 2013 [2], that subsequently spread across the Pacific to the Americas in a short timeframe [4] (*figure 1*). Nowadays, ZIKV is making headlines around the world, and the World Health Organization (WHO) has declared a public health emergency of international concern for this virus [5].

For more than sixty years, the early clinical picture of natural human ZIKV infection has mainly been associated with a self-limiting, mild febrile illness of short duration. However, like many other flaviruses, including yellow fever (YFV), dengue (DENV), and West Nile (WNV) virus [6], ZIKV has turned out to be a significant human pathogen. During the outbreak in Micronesia in 2007, ZIKV disease started to be associated with rash, high fever, arthralgia, and conjunctivitis, whereas during later outbreaks, several cases of Guillain-Barré syndrome were observed in French Polynesia, as well as meningoencephalitis in the Pacific Islands, and myelitis in Guadeloupe [7-9]. Most strikingly, the ZIKV epidemic in Brazil in 2015 has brought to light a temporal relation between fetal microcephaly and ZIKV infection of the childbearing mothers during the first trimester of pregnancy [10-12], prompting several national agencies to issue advisories to pregnant women and those considering pregnancy. Additionally, several case reports on sexual transmission of ZIKV [13], due to its persistence in semen [14] and vaginal secretions [15], have been published. Thus, unlike other flaviviruses, ZIKV is now characterized by its capacity of transplacental and sexual transmission, causing life-threatening neurological complications, that has highlighted its dangerousness (*figure 2*).

How did ZIKV, considered as an obscure and low-pathogenic mosquito-borne flavivirus for more than 60 years, emerge from its sylvatic forest existence in Africa and Asia to cause major epidemics throughout the Pacific and the Americas? Several not mutually exclusive possibilities have been proposed: First, with respect to the evolution of ZIKV strains, results from recent studies combining reverse genetics with mathematic models have provided evidence that ZIKV has acquired amino-acid substitutions around the same time as the detection of congenital Zika syndrome and other birth defects [16]. Second, it can be assumed that the intensification of the globalization process, associated with a modern lifestyle, could amplify an epidemic through travel of naive, non-naturally immunized individuals. Third, environmental elements, in partic-

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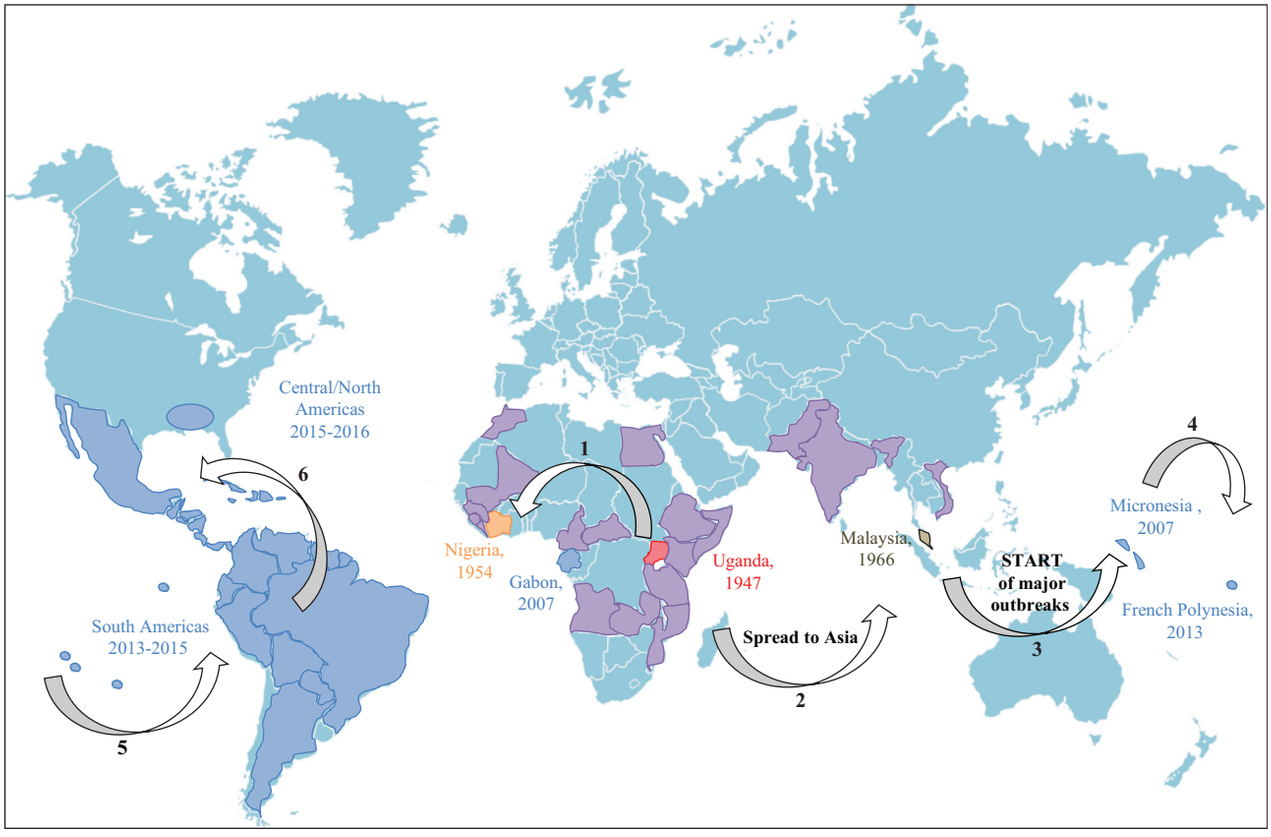

Figure 1

ZIKV spread from Africa to the Americas. (1) 1947: First documented in monkeys in Uganda. (2) 1960: First documented human cases in Nigeria. (3) 1970s: First cases in Asia. (4) 2007: Epidemic on island of Yap, Micronesia; (5) 2013-2015: Epidemic on French Polynesia and then through South America; (6) 2014-2016: ZIKV appears in northern Brazil and spreads through Central and North America.

ular climate change, are likely of critical importance for the survival and spread of mosquitoes.

Several mosquito species belonging to the Aedes genus have been identified as potential transmission vectors for ZIKV, and more especially *Ae aegypti* and possibly *Ae albopictus*, for their wide and increasing spread [17, 18]. As previously described for other flaviviruses, viral dissemination occurs via the skin, at the site of the mosquito bite, into the extracellular space of the dermis [19]. The initial step in the life cycle of flaviviruses is attachment of the virion to host-cell entry factor(s), including DC-SIGN, AXL, and TYRO3 that have been shown to be important for mediating ZIKV infection [19, 20], underscoring the pantropic nature of ZIKV. Following entry, ZIKV replicates in tissue macrophages and dendritic cells that traffic the virus to the draining lymph nodes and other lymphoid tissues. Increased number of macrophages is thus recruited that further amplify viral replication [21]. The "cytokine cascade" engaged during this early process of ZIKV infection will be discussed in this review.

THE "CYTOKINE CASCADE" IN ZIKA FEVER

The innate immune response is the first line of host defense against a viral infection. Multiple host pattern recognition receptors expressed on innate immune cells, including Toll-like receptors (TLR) and retinoic acid-inducible gene I (RIG-I)-like receptors, detect different pathogen-associated molecular patterns and trigger antiviral responses by producing type I interferons (IFNs), in particular IFN-β and multiple subtypes of IFN-α, that mount a rapid and potent innate defense against a number of viruses [22, 23]. Production of type I IFNs is initiated through recognition of pathogen-associated molecular patterns (PAMPs), generated during viral infection [24]. Binding of type I IFNs to their receptor, composed of two subunits (IFNAR1 and IFNAR2), activates the Janus kinases, Jak1 and Tyk2, as well as the signal transducers of transcription, STAT1 and STAT2, resulting in the upregulation of hundreds of IFN-stimulated genes (ISGs), whose activity restricts viral replication through a broad range of mechanisms [25]. The importance of type I IFNs in host antiviral innate immunity is highlighted by the diversity of viral strategies to evade these responses [26], some of which have been demonstrated for DENV, WNV, and YFV. It seems that the flavivirus nonstructural NS5 protein, which encodes both the viral methyltransferase and the RNA-dependent RNA polymerase, required for viral RNA synthesis, plays a key role in the inhibition of IFN-signaling by different mechanisms, depending on the flavivirus. WNV NS5 targets the host prolidase protein to prevent surface expression of IFNAR1 [27]. By contrast, YFV NS5 binds directly to STAT2, to prevent its binding to the IFN-stimulated responsive elements (ISRE) present upstream of ISGs [28], whereas DENV NS5 recruits the host E3 ubiquitin

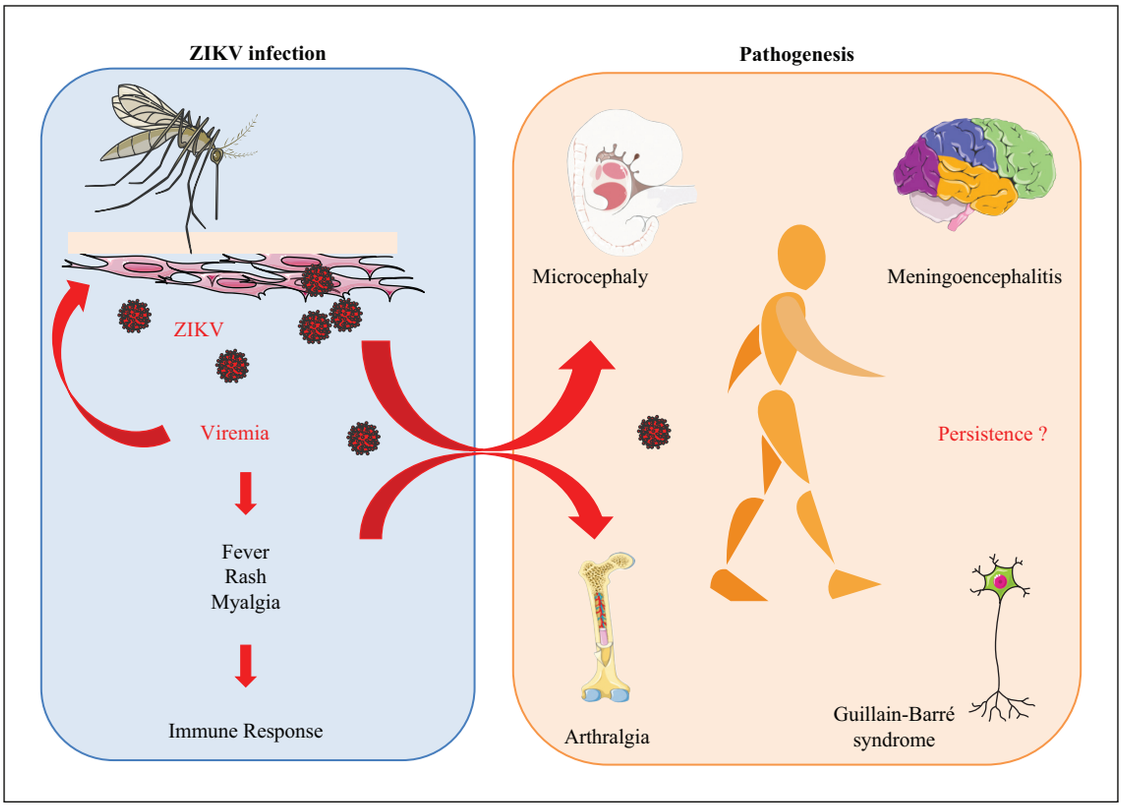

Figure 2
Virus dissemination, immune responses, and clinical manifestations in ZIKV-infected patients. ZIKV is transmitted through the bite of a female Aedes mosquito. Following infection of permissive cells in the dermis, such as endothelial cells, fibroblasts and macrophages and the virus replicates rapidly. Locally produced viral particles are transported through the circulatory system to secondary lymphoid organs, then disseminated to different organs. The acute phase of infection is associated with a high release of type 1 IFNs, followed by the up- or down-modulation of many other cytokines and chemokines, in association with the development of specific cellular immune responses. The infection by ZIKV can be accompanied by a spectrum of diseases, ranging from self-limiting meningo-encephalitis to congenital birth defects, like microcephaly, or Guillain-Barré syndrome.

ligase UBR4 to degrade STAT2 [29]. In ZIKV, NS5 expression results in proteasomal degradation of the IFN-regulated transcriptional activator STAT2, however, unlike DENV, via a UBR4-independent process [30]. This ability to strongly inhibit type I IFN responses has been proposed to favor a mild infection that allows flaviviruses to persist in the host and cause long-term defects [31].

As flavivirus NS5 proteins exhibit a remarkable, albeit virus-specific, functional convergence in their IFN type I antagonism, it is likely that ZIKV can also evade type III IFN (IFN-λ) signaling through STAT2 degradation via NS5. IFN-λ is a key cytokine produced abundantly at mucosal sites by epithelial and myeloid cells in response to viral infection [32]. Its induction and subsequent action at the epithelial layer of the vagina depends on the hormone-dependent stage of the estrous cycle [33]. Furthermore, IFN-λ protects trophoblasts, a layer of barrier cells in the human placenta from ZIKV infection [34, 35]. The ability of ZIKV to escape from the action of IFN-λ could contribute to its capacity to cross the placenta during pregnancy and cause neuronal disease in the developing fetus (see below).

Type I and type III IFNs are not the sole cytokines that are targeted by ZIKV. A longitudinal study from a Singapore cohort of ZIKV-infected patients revealed high levels of inflammatory cytokines and chemokines, such as GM-CSF, IFN-γ, IL-1ß, IL-2, IL-4, IL-5, IL-6, IL-9, IL-17, IL-22, CXCL10, CCL2, and CCL5, that were specifically identified in the acute phase of viral infection [36]. Most of these factors were previously described in two independent studies of Brazilian patients acutely infected by ZIKV [37, 38], making them useful markers for acute ZIKV infection. However, the over-expression of three of these pro-inflammatory mediators, IFN-γ, CXCL10, and CCL5, appears to be sufficient for generating an effective anti-ZIKV response, and consequently, a mild disease (*figure 3*). IFN-γ is unique in its action because it coordinates the transition from innate to adaptive immune responses by supporting macrophage activation and recruitment of other immune cells, like T_H1 lymphocytes, to the site of infection [39]. The expression of IFN-γ is limited to cells of the immune system and mainly to natural killer (NK) cells. These cells are a key element of the innate immune system, and represent a first-line defence against a variety of viral infections. They play both antiviral and regulatory roles via the release of soluble factors and operate via a balance of inhibitory and activating signals that enable them to detect and lyse virus-infected target cells [40-42]. To date, few data on the involvement of NK cells in ZIKV infection have been reported in the literature [43-45]. Our own data, obtained in a cohort of Gabonese patients, point to an early and transient

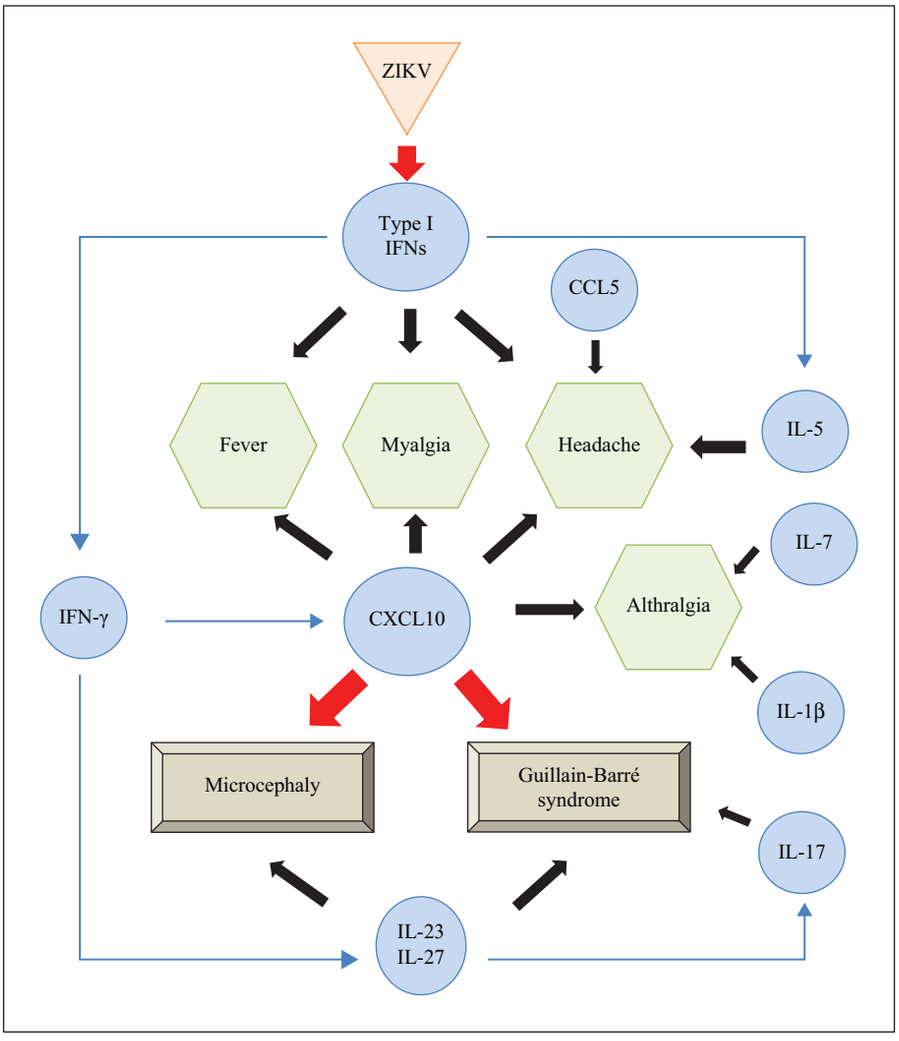

Figure 3
Model cytokine/chemokine pathways in the development of clinical symptoms mediated by ZIKV infection.

accumulation of specific NK cells, called adaptive NK cells, following an acute infection by DENV-2 that was absent in healthy donors [46]. This "clonal" expansion of adaptive NK cells, also described in other viral infections, such as Chikungunya, and cytomegalovirus [47, 48], is associated with a functional decoupling, in which IFN-γ production seems sufficient to control a DENV infection, in the absence of cytotoxicity (Petitdemange, Maucourant et al. Personal data). Upregulation of IFN-γ production by NK cells was also observed in WNV infection [49] and preliminary data suggested that NK cells from ZIKV-infected patients also produced high level of IFN-γ [43]. Interestingly, ZIKV infection has been reported to induce the expression of many IFN-γ-stimulated genes that, likely due to NS5-mediated depletion of STAT2, shift the STAT1-STAT2 balance toward STAT1, resulting in more STAT1 homodimers available to preferentially induce the transcription of IFN-γ-stimulated genes [50]. However, other groups have shown that pretreatment of human foreskin fibroblast with IFN-γ restricts ZIKV replication [19], possibly through the initiation of an inhibitory feed-back mechanism. Thus, the interplay between IFN-γ and ZIKV pathogenesis needs further investigation.

Monocytes, one of the first targets infected in the peripheral blood, also play a key role during the acute phase of infection by ZIKV, as highlighted by the high levels of GM-CSF and CCL2 produced early after infection [44]. CCL2 associated with IL-8 and CXCL10 was recently correlated with high viremia in symptomatic patients [36]. During the later phase of ZIKV infection, concentrations of IL-1ß, IL-2, and CCL2 in the sera, produced during the acute phase, were not decreased in ZIKV-infected patients during the later phase of infection, in contrast to DENV fever. However, in both infections, very high concentration of IL-10 was detected tardily [51-53]. It is of note that contrasting results were reported in these different studies for several other proinflammatory cytokines, like TNF-α and IL-6. Several hypotheses could explain these discrepancies, but the marked difference seems to be the studied populations. It seems that acute ZIKV infection in resident to an endemic area displays a modest proinflammatory systemic immune-activation profile, compared to non-residents individuals [38]. This suggests that constant exposure to ZIKV, and possibly other environmental factors, may affect the immunological inflammatory impact of infection. However, most studies to date have focused on direct

measurements of cytokines and chemokines in the peripheral blood compartment and have failed to interrogate the whole of the immune cascade in the context of the infecting pathogen and the rapidly changing immune environment in tissues.

In this context, it is also important to point out that immune cross-reactivity with other flaviviruses could be beneficial and result in cross-protection [54]; on the other hand, humoral cross-reactivity can also exacerbate disease through the process of antibody-dependent enhancement (ADE), of which DENV is the prototypic model [55]. Primary DENV infection results in a mild, acute disease with production of efficacious neutralizing antibodies, in which virus-antibody complexes are recognized by the Fc Receptor, internalized and destroyed. Problems may arise when a second DENV infection of a different serotype occurs, as the antibodies produced during the first infection can recognize and bind the second infecting strain, but with sub-neutralizing capability. For DENV, ADE has been associated with lower levels of innate immune mediators, such as nitric oxide or type I IFNs, and high production of IL-10 [55]. Whereas experimental evidence has demonstrated both a preventive, as well as a pathogenicity-enhancing role, of preexisting DENV antibodies in ZIKV infections, to date, ADE has not been confirmed in ZIKV [56, 57]. Because most countries with confirmed ZIKV cases are also endemic for DENV, there is a higher probability that ZIKV infection and immune response intensity may be amplified, owing to preexisting DENV cross-reactive antibodies; this should be a concern, particularly during vaccine development.

CYTOKINES ASSOCIATED WITH ZIKA-RELATED DETRIMENTAL NEUROGENESIS

Recent widespread outbreaks had brought ZIKV into spotlight, in particular because of the presumed causal relationship between infection and adverse fetal microcephaly. This has prompted the WHO to declare a Public Health Emergency of International Concern in February 2016 [58], and to advocate research into possible causal relationships and underlying mechanisms of ZIKV-induced neurologic disorders.

Fetal abnormalities

Pregnancy is a sophisticated biological process that relies on maternal-initiated immunosuppression toward the growing fetus particularly [59]. Because of the temporal and geographical overlap between the emergence of fetal microcephaly and the outbreak of ZIKV, the hypothesis was formulated that fetal microcephaly was caused by ZIKV infection during pregnancy [60, 61]. In the United States, there have been approximately 2,500 pregnant women infected with ZIKV and 116 infants born with ZIKV-associated birth defects since 2015. However, the majority of birth defects were reported in Brazil, which accounted for almost 400,000 cases of ZIKV infections and approximately 1,700 cases of neonates with confirmed microcephaly in 2015 [58].

Microcephaly results from any insult that disturbs early brain growth, and can be caused by genetic variations, teratogenic agents, or other congenital infections [62]. One potential mechanism for the observed microcephaly is the capacity of ZIKV to preferentially infect human neural progenitor cells and to trigger their apoptosis [63]. The capacity of the microglia to interact with ZIKV-infected tissues could also contribute to further spreading of the virus in the developing brain [64]. Activation of microglia leads to the production of pro-inflammatory cytokines, like TNF-α, IL-1β, IL-6, IL-12, and cytotoxic molecules, such as nitric oxide that aggravate inflammatory damage [65, 66]. In an extensive multiplexing analysis of 69 cytokines from a large cohort of pregnant women, high expression of CXCL10, in addition to CCL8 and CCL2, was associated with ZIKV-induced abnormal birth [67].

The majority of mediators modulated by ZIKV in pregnant women was also involved in the recruitment of monocytes and NK cells, like type I IFNs, IL-12, CXCL10, CCL8, or CCL2 [68, 69]. An excessive infiltration of both cell subsets at the maternal-fetal interface has previously been linked to pregnancy complications, such as preeclampsia and preterm birth [70, 71]. Instead, at the human implantation site, the predominant population of immune cells consists of uterine NK (uNK) cells and macrophages, which may comprise about 90% of all leukocytes, that are important for the control of placentation [72]. It is thought that the main function of uNK cells is to produce cytokines, such as TNFα, TGFβ, and IFNγ, as well as IL-1β and IL-10 [73]. Such production is regulated by inhibitory and activating receptors binding to HLA class I on trophoblast cells, but their role during ZIKV infection remains totally elusive. Interestingly, Foo *et al* [74] have recently shown that ZIKV infection promoted the dramatic expansion of nonclassical $CD14^{lo}CD16^{+}$ monocytes and an apparent production of the IL-10 in blood from the first and second trimesters of pregnancy; this cytokine produced by inflammatory monocytes and NK cells is known to promote viral persistency and to dampen host defenses [75], and can be detected at high level in the amniotic fluid of pregnant ZIKV patients who had microcephalic fetuses or neonates [76]. Production of IL-10 is certainly a marker of a counter anti-inflammatory response that has been termed "immunoparalysis." Downregulation of systemic inflammation by IL-10 in ZIKV may be conceptually beneficial in controlling systemic responses to local infection, but also detrimental with the development of fetal abnormalities in ZIKV-infected pregnant women.

Together, these observations suggest that excess production of certain factors, like IL-10 and CXCL10, driven by specific monocytes and uNK cells in $ZIKV^{+}$ pregnant women could contribute to neuronal damage affecting the developing fetal brain and the development of microcephaly.

Guillain-Barré syndrome

Convincing evidence has also associated ZIKV infection with the development of Guillain-Barré syndrome

(GBS), an infrequent autoimmune disorder characterized by progressive muscle weakness of limbs and areflexic paralysis [77]. GBS is the most common cause of neuromuscular paralysis and, in rare cases, may lead to death. Its worldwide incidence is approximately 1 case per 100,000 people. The first challenge to the apparently benign nature of ZIKV infection occurred during the outbreak in French Polynesia, which began in October 2013, when 45 individuals developed GBS [78]. In 2015, the WHO reported 1708 cases of GBS in Brazil, although this must be interpreted with caution because several cases were not tested for ZIKV infection [79]. The reasons for the increase in the incidence of GBS in Brazil, but also El Salvador, and Suriname are unknown, particularly because potentially other viral pathogens might be involved in particular DENV or CHIKV that are co-circulating in these countries, during the same period. Importantly, GBS associated with ZIKV infection was found to be associated with a higher morbidity during the acute phase, compared with GBS triggered by other etiologies [66]. This suggests that the immune response specific to ZIKV infection could be partially implicated in the symptomology of the GBS.

Pro-inflammatory cytokines play various roles in the pathogenesis of GBS, such as TNF-α, IFN-γ, IL-1β, IL-6, IL-12, IL-17, IL-18, and IL-23, but also anti-inflammatory mediators, like TGF-β, IL-4 and IL-10, as well as IL-27, that exerts both pro-inflammatory and anti-inflammatory effects, [69, 80]. It seems that IL-23 and IL-27, two members of the IL-12 family, are more particularly associated with the recovery of GBS [81], whereas CXCL10 has been implicated in GBS pathogenesis [82] (*figure 3*). Thus, it was hypothesized that high levels of CXCL10 in ZIKV patients may contribute to neuronal damage affecting the developing fetal brain and potentially targeting peripheral nerves in Guillain-Barré syndrome as well [66].

CONCLUDING COMMENTS

Across the world, infectious diseases remain a real threat, accounting for approximately half of all deaths each year. Tuberculosis, malaria, AIDS, influenza, as well as endemic and (re)emerging flavivirus infections, like ZIKV, all contribute to morbidity and mortality. Economic development, urbanization, and environmental degradation gather pace, whereas the structure of societies changes, creating a "perfect storm" for the future spread of ZIKV, leading to new challenges in the future. Against this backdrop and the absence of an effective vaccine against ZIKV infection, although actively sought [83, 84], increasing interest has focused on the development of drugs that target the cytokine response following ZIKV infection. The virus/host interaction is a complex interplay between pro- and anti-viral components that ultimately determines the spread or halt of virus infections in tissues. Integrating the data listed above in this review reveals the role for certain key cytokines in the pathology of ZIKV (*figure 3*). High production of CXCL10 and IL-10 is associated with several aspects of ZIKV-related detrimental neurogenesis, including microcephaly and/or GBS. Thus, it should be important to try to target these mediators in order to reduce the collateral damage initiated by the host immune response to ZIKV. As previously shown, CXCL10 neutralization by specific antibodies or genetic deletion in $CXCL10^{-/-}$ mice protected against cerebral malaria infection and inflammation [85], and passive transfer of anti-CXCL10 antibodies reduced inflammatory leukocyte recruitment across the blood-brain barrier. Furthermore, statin medications commonly used for cholesterol control have been shown to decrease CXCL10 and to be effective in Crohn's disease [86, 87]. However, to date, successful targeting of the immune system during an acute infection has proved to be extraordinarily difficult and largely unsuccessful. A reason could be that we still do not totally understand the delicate nature of the rapid changes of the cytokine response during an acute infection, and until we do, it is unlikely that we will be able to develop rational therapies that target the exact phase of the immune cascade and administrate those therapies at the time they are needed.

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