Zika virus (ZIKV) is an arbovirus that is transmitted by Aedes mosquitoes. Its prototype was isolated in 1947 from serum of a sentinel Rhesus monkey (Macaca mulatta) in the Zika forest of Uganda. As a member of the genus Flavivirus, family Flaviviridae, ZIKV is enveloped and icosahedral and possesses a single-stranded, positive-sense RNA genome of approximately 10.7 kb. Epidemiologically, infection by ZIKV has become a global health concern in recent years because of the occurrence of epidemics, its speed of dissemination, routes of transmission, and the sequelae it can cause especially in newborns. At the neural level, there are still many gaps in our understanding of the mechanisms that induce ZIKV infection-associated microcephaly. However, some studies already demonstrated that underlying cell death is determinant to induce the congenital malformation. In this report, we reviewed the various mechanisms of cell injury involved in the immunopathogenesis of ZIKV infection and discussed its relationship with the death of neuronal and glial cells development and microcephaly.

**Keywords:** ZIKV, microcephaly, neuroinflammatory response, cell death

**Perspectives**

Zika virus (ZIKV) is a member of the family Flaviviridae and the genus Flavivirus; it was originally isolated in 1947 from a febrile sentinel rhesus monkey in the Zika forest (Uganda).<sup>1</sup> In 2015, the World Health Organization (WHO) declared the Zika epidemic a public health emergency in Brazil owing to the outbreak and exponential growth of reported cases of microcephaly and other congenital defects.<sup>1,2</sup>

ZIKV exhibits extreme versatility and modulates mechanisms of immune evasion; recent experimental advances have shown that ZIKV infects different cell types in several organs and tissues, confirming that transmission occurs not only by bites of infected mosquitos (*Aedes*), but also by sexual and vertical routes.<sup>3</sup> In this context, it is believed that receptors belonging to the TIM and TAM families, particularly AXL, facilitate ZIKV entry in cells.<sup>4</sup>

Considering the concept of immune privilege and the high selectivity of the blood-placental, blood-testis, and blood-brain barrier to control protein and immune cell entry in the organs, the immune responses mediated by dendritic cells, M1/M2 macrophages, endothelial cells, and CD4 and CD8 lymphocytes reflect the complexity of the host defense system and its organization to combat infectious agents.<sup>5–7</sup>

Recent experimental studies in vitro and in vivo have shown that several mechanisms of cell injury are involved in the immunopathogenesis of ZIKV infection. These studies suggest that there is a link between microcephaly and...
Both necrosis and apoptosis are 
classic apoptosis leads to a process of natural 
and autophagy increase the death of neuronal and 
glial cells and, consequently, result in brain atrophy and 
ZIKV-induced microcephaly.

**Apoptosis, Necrosis, And
Necroptosis**

The development of microcephaly presents a great chal-

lenge owing to the lack of a comprehensive understanding 
of the evolutionary mechanisms and dynamics of the pro-
cess. Accordingly, further studies are needed to clarify 
these issues and to determine the mechanisms underlying 
cell injury. A loss of tissue homeostasis and thereby a cell 
functional imbalance can result in the induction of necrosis 
or apoptosis pathways. Both necrosis and apoptosis 
are observed in cases of ZIKV-induced microcephaly.

More recent investigations showed the involvement 
of components of the necroptotic cell death pathway to limit 
novel neuronal Zika virus infection. In cortical neurons, 
the activation of Z-DNA-binding protein 1 (ZBP1) and 
RIPK1/RIPK3 can trigger the formation of intracellular 
cascade which modulate the expression of immunorespon-
sive gene 1 (IRG1), as well as itaconate metabolite. In this 
process, itaconate inhibits the formation of succinate dehy-
drogenase (SDH) and consequently suppresses the replica-
tion of viral genomes.

With respect to apoptosis, extrinsic and intrinsic path-
ways act jointly to enhance cell damage. In the extrinsic 
pathway, cytokines, growth factors, and death ligands induce 
the activation of death receptors responsible for initiate 
the intrinsic cascade. Activation of the intrinsic cascade results in 
mitochondrial damage that alters the electron transportation 
chain, provokes the formation of pores in the membrane 
where cytochrome C is released to bind with Apaf1, and 
forms the apoptosome complex responsible for activating 
a series of caspases that direct the cell to apoptosis. This 
process involves the caspases 6, 7, 8, 9, and, in particular, 
caspase 3, which mediate programmed cell death induced by 
ZIKV. Classic apoptosis leads to a process of natural 
cell death in which the host defense system induces various 
mechanisms to maintain homeostasis, without causing major 
damage; in microcephaly cases, apoptosis induces the uncon-
trolled death of neurons and glia cells at several stages.

This has direct relationship with cellular cycle as evidenced in 
study cell death showing that in the ventricular zone, 
progression and mitotic abnormalities of progenitor neural 
cells in the brain are associated with the intense production of 
caspase 3, 7, 8, and 9.

**Pyroptosis**

Dynamic, interacting processes are linked to the immune 
response, and recent study of cell death mechanisms has 
shown that endoplasmic reticulum stress directly contrib-
utes to the activation of signaling proteins that modulate 
excitotoxicity, increase intracellular calcium production, and 
enhance cell damage. In the CNS, an intense neuroinflam-
matory response and loss of mitochondrial homeostasis 
results in the development of a new cell death mechanism 
that is extremely harmful to neurons and glial cells, i.e., 
pyroptosis in microcephaly cases ZIKV-induced.

Interestingly, pyroptosis differs from apoptosis and necrosis 
since it is dependent on the activation of the inflammasome, 
a multiprotein complex characterized by the activation of 
NLRP3 and AIM2, receptors that recognize pathogen-asso-
ciated molecular patterns (PAMPs) of the ZIKV and induce 
the release of caspase 1 to activate pyroptosis and trigger 
the maturation of IL-1β, IL-18, and IL-33. Studies of these 
cytokines are necessary to understand the neuroinflam-
matory mechanisms involved in the development of microcephaly; 
IL-1β, IL-18, and IL-33 belong to the IL-1 family and modulate the activities of the M1 (IL-1β and 
and M2 (IL-33) macrophages/microglia as well as the Th1 (IL-1β and IL-18) and Th2 (IL-33) lymphocytes.

Recently in experimental in vitro was showed that ZIKV 
reserve proinflammatory response to promote NLRP3 

inflammasome activation by stabilizing caspase-1 to sup-
press cGAS-mediated type I IFN signaling. In this context, 
NS1 block the proteasomal degradation of the caspase 1 
recruiting USP8 to cleave K11-linked poly-ubiquitin chains 
from caspase-1 at Lys134. The implications of this process 

enhance inflammasome activation and inhibit type I IFN 
signaling.

**Paraptosis**

As an immune escape mechanism and adaptation, ZIKV uses 
the cellular machinery to transform the cell into a setting for 
viral replication, inducing massive cytoplasmic vacuoliza-
tion in human epithelial cells, primary human fibroblasts, 
and human astrocytes. This process is associated with a 
new form of cell death that does not result in apoptotic corpuscles, known as paraptosis. The mechanisms under-
lying paraptosis in cells of the CNS involve PIK3, AKt, 
PERK, IRE-1, and ATF-6, as well as vesicle transport or-
chestrated by the endoplasmic reticulum and Golgi
Interesting that ZIKV-induced vacuoles are dependent on viral translocation into ER through Sec61 and on PI3K/Akt signaling. Therefore, the paraptosis makes the cell a dangerous ZIKV reservoir via the massive cytoplasmic vacuolization, functional alterations of the endoplasmic reticulum, delayed phagolysosome maturation, and the excessive production of ROS; accordingly, it can contribute to cell destruction and ZIKV dissemination.

**Autophagy**

In addition to paraptosis, another cell death pathway implicated in the pathogenesis of ZIKV-infection is autophagy. Autophagy regulation in the brain is associated with ZIKV tropism by several CNS cells. In vitro the infection of human fetal neural stem cells (fNSCs) with ZIKV leads to impaired neurosphere formation and elevated autophagy. Infection with ZIKV strains MR766 and IbH30656 efficiently induced LC3-I to LC3-II conversion and LC3 puncta formation of fNSCs in the presence or absence of lysosome inhibitor bafilomycin A1. Interesting that in this process two ZIKV proteins (NS4A and NS4B) inhibit Akt and consequently the PIK3 cascade and mTOR production causing the deregulation of neurogenesis aberrant activation of autophagy. Curiously this both proteins produced by dengue virus do not have the same effect on neurogenesis. Therefore, the identification of this mechanism suggests a strong actuation of NS4A and NS4B to modulate the immune evasion of ZIKV in the viral pathogenesis.

**Conclusion And Future Direction**

Many questions about microcephaly induced by ZIKV remain. However, investigations of several mechanisms of cell injury involved in the immunopathogenesis of ZIKV infection suggest that there is a link between microcephaly and cell death in which the neuroinflammatory response in situ can induce deleterious effects in vitro in neurosphere leading human fetal neural stem cells (fNSCs) to cell death.
apoptosis, necrosis, and pyroptosis and consequently result in brain atrophy. In vitro triggering of the necroptosis, parapoptosis, and autophagy enhance death of neuronal and glial cells. Therefore, identification of these mechanisms enables the discovery of several immune-targets for anti-ZIKV therapeutic intervention. However, we emphasize that studies in vivo need to be performed to confirm this hypothesis. In summary, we propose an integrated model that synthesizes the relationships among these underlying mechanisms (Figure 1).

Funding
This work was supported by the Ministry of Science, Technology and Innovation/National Council for Scientific and Technological Development CNPQ/Brazil (grant numbers: 303999/2016-0, 439971/2016-0, and 440405/2016-5), and CAPES (Zika Fast-track).

Author contributions
All authors contributed towards data analysis, drafting and critically revising the paper, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work.

Disclosure
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References