Chapter

Innate Immunity Modulation during Zika Virus Infection on Pregnancy: What We Still Need to Know for Medical Sciences Breakthrough

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Abstract

Zika virus (ZIKV), an arthropod-borne flavivirus, was classified as reemerging infectious disease and included as neglected tropical disease. During the recent ZIKV outbreak in South America, it has been demonstrated that ZIKV infection during pregnancy is strongly associated with fetal loss, malformations and neurological disorders in newborns. Despite the first line of host immune defense is related to innate immunity activation, the immunological homeostasis is essential for pregnancy success. Although the dynamic changes in maternal-fetal immunity is not completely understood and poorly investigated, the knowledge of immune responses during gestation is very important for infectious disease prevention and control, as ZIKV. Here, we put together more and new information about the innate immunity during gestation, highlighting three parts probably involved with clinical outcome and/or not well explored in literature: 1) type III interferon; 2) innate regulatory cells; and 3) cell death pathways modulation. Additionally, we will be focused on discussing how the dynamic responses of innate immune system during pregnancy and its effects in newborns, could be modulated by ZIKV, as well as how efforts on development of new/old drugs and vaccines could be effective for ZIKV prevention and control to provide a successful pregnancy.

Keywords: innate immunity, pregnancy, zika, technological development

1. Introduction

Zika virus (ZIKV) is an arthropod-borne flavivirus, considered a reemerging infectious disease as well as a neglected tropical disease [1]. Moreover, ZIKV was also classified as sexually transmitted disease (STD), since viral RNA and infectious particles were detectable in reproductive organs and others described some cases related to sexual transmission [2, 3]. Although the major concern about ZIKV infection is the intrauterine transmission [4–6].

Innate immunity during pregnancy still needs attention when some infection compromises pregnancy success. Recently, the world testified a huge public health problem during Zika virus (ZIKV) outbreak in Latin American countries [7–9], in which poor outcomes were observed firstly in Brazilian newborns from mothers infected on early pregnancy phase (1st -2nd trimester) [7, 8]. Consequences of viral infections on newborns are irreversible and public health and social costs are immensurable [10], making World Health Organization consider Zika infection a public health emergency in 2016 February [11].

Due to its neurotropic features, the infection caused by ZIKV has been evidenced [12–14], which show a correlation between clinical manifestations based on its tropism by brain neuronal cells of fetuses and neonates born from infected pregnant women, with a strong association to neurological damage, including microcephaly and other fetal neurological disorders, collectively named as Congenital Zika Syndrome (CZS) or Zika Associated with Birth Defect (ZABD) [15–18].

The immune system is composed of a set of flexible mechanisms that are fundamental to maintain homeostasis, allowing many interactions and coexistence between different populations of microorganisms and the host. The imbalance of homeostasis can be caused by a microorganism because of its pathogenic behavior. With the establishment of an active infection and consequent immune response, inflammatory mediators, produced initially, collaborate to activate cellular populations of the innate immunity, promoting antiviral and cytotoxic responses, for example. At first, these effector responses would influence the viremia resolution with the re-establishment of homeostasis. However, the loss or dysfunction of this immune response can generate a harmful environment that triggers an uncontrolled damage inflammation and consequent cell death due to a direct cytopathic effect caused by the microorganism [19].

Some studies were conducted to understand the mechanisms involved in vertical transmission. During pregnancy, the transfer of ZIKV to the placenta occurs after an infection of decidua, the placenta maternal region, since studies have shown that decidua cells are permissive to ZIKV infection and remain permissive throughout pregnancy [20, 21]. From the infection of the decidua, there are two routes by which ZIKV reaches the fetus: infection of syncytiotrophoblasts (SBTs) through capillaries containing maternal blood or infection of Extravilous Trophoblast (EVTs) by cell-to-cell propagation [4]. In vitro studies have shown that ZIKV can infect first-trimester cytotrophoblasts CTBs and EVTs [4, 20, 21]. On the other hand, STBs are high producers of type III interferon and remain relatively resistant to viral infection throughout pregnancy, therefore, the main route hypothesis for transplacental transmission of ZIKV is that of the spread of decidua to EVTs [21, 22]. Additionally, infection of placental macrophages, the Hofbauer cells by ZIKV may contribute to both intrauterine transmission and immunomodulation [23, 24]. Further, transplacental transfer of ZIKV is more likely to occur in the pro-inflammatory environment and tolerant to placental immunity in the first trimester.

Histopathological and immunological studies in placentas have shown that infections by ZIKV lead to an increase in important inflammation markers such as TNF, CCL5, and altered vascular permeability such as metalloproteinases [25]. In addition, in vitro experiments demonstrate that trophoblastic cells become progressively more resistant to infection by ZIKV during pregnancy, partly through the secretion of IFNs [26]. In this context, a lot of efforts were raised to provide funds to deeply investigate how to avoid another spread of Zika virus infection, as well as drugs tests and vaccine development based on viral proteins, DNA vaccines, Virus Like Particles (VLP), chimeric viruses, among other strategies [27–30]. Therefore, there are few studies to investigate the pregnancy immunity and how the immune interface mother-to-child could contribute to infection spread with drastic

consequences to fetus [21, 31–34]. To our knowledge, the imbalance of normal pregnancy immunity is already cause of metabolic disorders and the poor outcome is related to abortion [35–37]. Then, a viral infection can make this picture worst and tragic [8, 13, 15, 38, 39].

Like other Flaviviruses, ZIKV life cycle modulates machinery and functions of target immune host cells, making essential virus-cells interactions for pathogenesis development. Moreover, while several human and animal models' studies have argued and proved ZIKV neurotropism, there are still many answers regarding viral pathogenesis in mother and its influence the fetal neural system and persistence, and clinical outcome. In this chapter we will put together the information about innate immunity during gestation, highlighting three parts probably involved with clinical outcome: 1) interferon type III; 2) innate regulatory cells; and 3) cell death pathways modulation. Additionally, we will focus on discussing how the dynamic responses of innate immune system during pregnancy and its effects in newborns, could be modulated by ZIKV, as well as how efforts on development of new/old drugs and vaccines could be effective to help pregnancy success.

2. Type III interferon

The success of pregnancy is dependent on a coordinated balance between the "invading" fetal trophoblast and a receptive maternal decidua in the placenta, maintaining a dynamic and responsive immune system. The longest period of the pregnancy, fetal growth, demands a symbiotic and tolerogenic environment, but congenital viral infections can disrupt this equilibrium. In order to avoid infection severity placenta actively modulates the immunologic profile of the maternal-fetal interface [40, 41]. In this context, recent studies demonstrated that placenta responds to ZIKV infection by production of the newest interferon group type III interferons [21, 42, 43].

Type III interferon (IFN-λ 1–4) comprising a group of cytokines with action pathways under strengthen discovery [44–46], basically acting with shared inflammatory regulation and antiviral properties [47]. IFN-λs receptor was identified as a complex composed of two subunits: IFN-λR1 and IL-10R2, which is also a receptor subunit of the regulatory cytokines IL10, IL22, and IL26 [48]. In contrast with the classical pro-inflammatory type I interferons which receptors are expressed in almost all cell types, the IFNLR1/IL10RB complex is expressed primarily in cells of epithelial origin and few immune cells conferring selective IFN-λ responsiveness to them: neutrophils [49], myeloid dendritic cells (DCs) [50, 51] and plasmacytoid dendritic cells (pDC) [52]. Because of the restricted cell types producing IFN-λs, this cytokine acts locally as an immunologic barrier in organs with suppressing innate pro-inflammatory responses and limiting host damaging effects associated with inflammation [53]. Moreover, IFN-λs utilize mechanisms to suppress viral infections which induce a strong antiviral state following receptor binding with non-translational and translational processes [49, 54].

Between the different inflammatory regulation actions already described for IFN-λs, the suppression of neutrophil gains prominence because they are the immune cells that present higher expression.

of IFN-λR1 at the steady-state [55–57]. Neutrophils contribute to various stages of the reproductive process since conception and implantation, ensuring fetal wellbeing during pregnancy and finally contributing to parturition and postpartum maternal health. On the other hand, aberrant neutrophil activity is associated with severe pregnancy-related disorders such as pre-eclampsia, recurrent fetal loss or gestational diabetes mellitus [58–60]. In murine models, it was demonstrated

that neutrophil exposed to IFN-λ can induce antiviral interferon-stimulated genes (ISGs); and IFN-λ (but not IFN-β) specifically activated a translation-independent signaling pathway that diminished the production of reactive oxygen species and degranulation in neutrophils, which might permit a controlled development of the inflammatory process [49].

Studies utilizing a cellular model of collagen-induced arthritis demonstrated that IFN-λ2 was protective and could stop the progression of the disease, diminishing infiltration of neutrophils to the inflamed joints as well as the production of IL-1β upon treatment with pegylated recombinant IFN-λ2 [57]. *Ex vivo* experiments with cardiopathic patients` cells demonstrated that IFN-λ inhibits Neutrophil Extracellular Traps (NETs) [61]. NETosis has been appointed as critical agents during pregnancy, particularly involved an auto-inflammatory process involving the release of placental micro-debris in preeclampsia and recurrent fetal loss [62]. In collagen-induced arthritis murine models, it was demonstrated that IFN-λ exerts its anti-inflammatory effect by restricting recruitment of IL-1β–expressing neutrophils, which are important for amplification of inflammation, and reducing IL-17–producing Th17 and γδ T cells in the joints and inguinal lymph nodes, without affecting T cell proliferative responses [57].

IFN-λ is strongly associated with DCs activity inducing an effector adaptive immunity response [63, 64]. Studies with a mice model of influenza A virus infection demonstrated that IFN-λ directed acts in the migration and function of CD103(+) dendritic cells, also regulating DC IL-10 network [65]. Migratory CD103(+) DCs derived from skin, lung, and intestine, efficiently present exogenous antigens in their corresponding draining lymph nodes to specific CD8(+) T cells through a mechanism known as cross-presentation, demonstrating the IFN-λ importance for the development of specific CD8+ T cell responses [65, 66]. Moreover, IFN-λ contributes to the formation of tolerogenic DCs cell, contributing to control inflammatory responses and homeostasis by fostering the conversion of naive T cells into induced Foxp3(+) regulatory T cells [66]. In vitro studies demonstrated that IFN- λ directs DCs to a regulatory phenotype with diminished capacity to stimulate T cell proliferation in a PD-1/PD-L1 dependent manner with contribution from the imbalanced cytokine milieu, such as low IL-12 and IL-2 and/or high IL-10 production [50]. Another study using mixed lymphocyte cultures demonstrated that IFN- λ -treated DCs specifically induced IL-2-dependent proliferation of a CD4(+) CD25(+) Foxp3(+) T-cell subset with contact-dependent suppressive activity on T-cell proliferation initiated by fully mature DCs [51].

Plasmacytoid dendritic cells (pDC) are rare cells found in peripheral blood and lymphoid tissues, considered to be "professional" type I IFN-producing cells and produce 10- to 100-fold more IFN-α than other cell types in response to enveloped viruses. However, in vitro IFN-λ treatment of pDC resulted in increased virus-induced expression of both IFN-α and IFN-λ, indicating that pDC are high producers of IFN-λ1 and -λ2 in response to viral stimulation and the consequences of this high IFN-λ production by pDC should be further explored [52].

In human congenital ZIKV infections, it was demonstrated that ZIKV infection leads to a typical inflammatory response in the placenta, including the expression of anti-viral Type I interferon genes (*IFIT5*, *IFNA1*, and *IFNB*), type II interferon (*IFI16*), cytokine signaling (*IL22RA* and *IP10*), and interferon regulatory factors (*IRF7* and *IRF9*). Furthermore, the CZS cases present a gene expression profile with impaired *IFNL2* response, accompanied by an exacerbated type I IFN response; with an increased expression of *IFIT5*, parallel to a decrease in *ISG15* mRNA [67], which was already identified as negative modulator of type I IFN and protective against ZIKV ocular manifestations [68]. These results are corroborated by *in vitro*

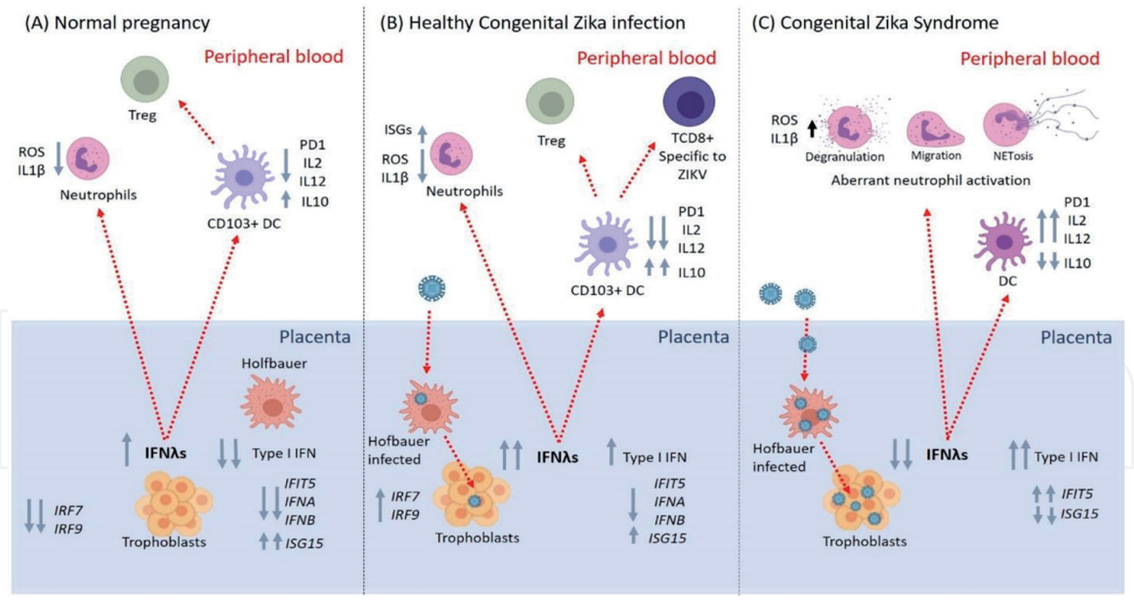

Figure 1.
Summary of Interferon lambda (IFN-λ) function during normal pregnancy (A), Healthy Congenital Zika infection (B), and Zika-Associated Birth Defects (C). (A) In normal pregnancy, trophoblasts exhibit a constitutive IFN-λ production, contributing to the general tolerogenic environment demanded by pregnancy (A1); Considering the peripheral blood tissue IFN-λ Interact with: (A2) neutrophils leading to a decrease in ROS and IL1β, and (A3) migratory CD103+Dendritic cells (DC) that present low levels of PD1, IL2 and IL12 together with high IL10. These CD103+DC foster the conversion of naive T cells into induced Foxp3(+) regulatory T cells (Treg) (A4). In the placenta, the constitutive IFN-λ is accompanied by decreased type I IFN pathway: low expression of IFIT5, IFNA, and IFNB, and high expression of type I IFN the negative regulator ISG15 (A5). In the lack of viral infection, the interferon regulatory factors IRF7 and IRF9 present low expression levels (A7). (B) In healthy congenital Zika infections, the placenta expresses high levels of IFN-λ to protect the fetus from congenital defects (B1). In this low damage antiviral response, high levels of IFN-λ elicits the production of ISGs and the decrease of ROS and IL1β by circulating neutrophils (B2), meanwhile the CD103+ DC presents an accented regulatory profile (B3), with induction of high specific anti-ZIKV response by Treg (B4) and TCD8+ cells (B5). In the placental level type, I interferon pathway shows a slight increase, together with the enhance of IRF7 and IRF9, forming a balanced antiviral response. (C) In Congenital Zika Syndrome (CZS) the lack of IFN-λ contributes to a damaging outcome (C1). Diminished levels of IFN-λ could not control the neutrophil activity, culminating in augmented ROS and IL1β (C2), and presence of aberrant activation forms as well as degranulation, migration, and NETosis (C3). Without IFN-λ the Dendritic Cells (DC) present a pró-inflammatory profile, with augmented PD1, IL2, and IL12 and diminished IL10 (C4). The placenta shows an exacerbated type I interferon response, which together with low IFN-λ levels (C5), leads to an imbalanced damaging antiviral response. Grey arrows represent the production or expression levels (up = high, down = low). Double arrows represent a high magnitude of production or expression. Red dashed arrows represent the direction of function/induction events that have been known and those suggested. Figure created using Biorender software (https://www.biorender.com).

studies that showed induction of *IFNL1* expression by susceptible placental cells after ZIKV infection, acting as an antiviral agent [43], reinforcing that IFN-λs are protective factors in ZIKV congenital infections. Studies with *ex vivo* placental 3D cultures from a different trimester of healthy pregnant volunteers showed that IFN-λs are expressed mostly by deciduous (the maternal portion of the placenta), already indicating that mothers are the agents on the immunoregulation of CZS outcome (**Figure 1**) [21].

3. Innate regulatory cells - myeloid-derived suppressor cells (MDSC)

Immunity during pregnancy is very important to be explored since successful pregnancy requires that immunoregulatory mechanisms are triggered to suppress activated fetal-specific T cells lymphocytes [36, 37]. Maternal immune cells can recognize paternal antigens on fetus. Thus, it has been very well described that

dysfunction of immune cells during pregnancy can lead to immunologic fetal rejection by mother, in which the consequences are related to abortion, preterm delivery, or other severe complications [35–37].

Then, maternal-fetal tolerance involves the regulation of mother's immune system to tolerate the semi allogeneic fetus expressing paternal antigens without immune rejection. Even though, some studies showed that regulatory T cells are the main cells which plays an important role in suppressing activated T cells during gestation; since then innate immunity system is poorly investigated [69–71].

Considering infections during pregnancy, it is also important to know that changes on maternal immune responses are required to induce limited immunosuppression without loss of host defense, in which a balance between activated and immunosuppressed cells needs to be regular [35].

Myeloid-derived suppressor cells (MDSC) are a heterogeneous mixture of immature myeloid cells, been part of innate immune cells, having a crucial role in immunomodulatory mechanisms during pregnancy [36, 72, 73]. There are two subtypes of MDSC, a monocytic and granulocytic. Phenotype is characterized by expression of CD33 and CD11b in humans, CD14 by monocytic MDSC and CD15 by granulocytic MDSC cells but lacks the maturation marker HLA-DR. But both subtypes share the characteristic of immune-suppressive function inhibiting activated NK and T cell expansion [73, 74].

Normally, immature myeloid cells as MDSC are scarcely found in peripheral blood, and their maturation includes macrophages, dendritic cells, and granulocytes formation. Nevertheless, the MDSC are also recognized by their role in some pathological conditions, like cancer, sepsis, stress, autoimmune disorders and infectious diseases [38, 75, 76].

Several studies have been reported that a decrease of MDSC during pregnancy may lead to poor outcomes, as miscarriage [77]. Also, it has been shown that progesterone levels increase MDSC during pregnancy in mice, as well as high levels of TNF and IL-1β, pro-inflammatory cytokines [38, 78].

In murine models, it was demonstrated that MDSC can produce TGF-β and IL-10, as immunosuppressive cytokines, similarly to regulatory T cells. Adding to that, MDSC can suppress T cell activation and function by arginase-1 (Arg-1) secretion, as well as nitric oxide synthase and indoleamine 2,3 dioxygenase aimed to deplete nutrients for T cell proliferation, as I-arginine (I-Arg). According to Ismail 2018, arginine is also involved in replication, and virulence of several agents, as viruses and bacteria. Then, it is suggested that an accumulation of MDSC in placenta could influence an increase of arginase activity, and it would serve for a dual purpose, inhibiting the adaptive immune system whilst also providing potential protection against infection by arginine auxotrophic pathogens [79].

Nitric oxide (NO) has been related to embryo successful implantation during early pregnancy, but excessive NO production by decidual macrophages seems to be harmful and was linked with early pregnancy loss [37, 80, 81]. Another study suggests that in early pregnancy in decidua CD33+ cells express nitric oxide synthase, playing an important role to maintained pregnancy during this phase, while in later pregnancy CD33+ cells lose the expression of this enzyme [35, 37].

Kostlin-Gille *et al* 2019 showed that hypoxia condition is important to normal placenta development and its driven by a hypoxia-inducible factor 1 (HIF-1), a key regulator responsible for initiate transcription of several genes. The subunit HIF-1α is highly expressed in placenta during early gestation period, characterized by low oxygen pressure conditions. This study used myeloid HIF-1 knockout mice to evaluate the role of HIF-1α on myeloid-derived suppressor cell function, showing that HIF-1α deficiency in myeloid cells leads to diminished suppressive activity of MDSC in uterus from pregnant mice, but the expression of chemokine receptor or

integrins was not altered. Despite MDSC recruitment to uterus was not altered, it was observed a lower MDSC accumulation as well as an increase of MDSC apoptosis, contributing to an elevated abortion rate in knockout mice [73].

Regarding Zika virus, there are few studies showing the presence of MDSC on women blood and during pregnancy, and considering the facts, it will be very important to know any relationship of their presence with congenital syndrome, as observed in 2016, Brazil [82, 83]. A study with 10 non-pregnant women with Zika infection showed that frequencies of circulating MDSC did not change over time [84]. Another study with pregnant monkeys infected with Zika virus showed that an imbalance on blood frequencies of MDSC and activated CD8 T cells in the acute phase may lead to poor outcome to the fetus. Adding to that, the high frequency of MDSC on placenta from pregnant monkeys showed a positive effect on pregnancy outcome, even more if a drug antiviral treatment was used [85].

Furthermore, it is worth to note that immune signature, sometimes is the key factor to explain some diseases progressions. Despite Dengue viruses is more related to signals and symptoms with Zika virus infection [86, 87], some similarities with hepatitis C virus (HCV) were also noted, and mechanisms of immune evasion have been described, as inhibition of interferon pathway, allowing virus life cycle for a long-term period, up to 100 days [88, 89]. To note, ZIKV infection is also classified as an immune-mediated viral disease, like Dengue and other viruses [86, 87, 90]. Disease progression in HCV patients to chronic infection has been associated to an increase of MDSC phenotype in peripheral blood mediated by viral proteins [38]. Wang et al., 2017 examined Japanese encephalitis virus (JEV) infection leading acute encephalopathy depending on suppression of adaptive immune response, especially T follicular helper cells, mediated by enhanced MDSC populations, such as an involvement of MDSC on splenic B cells reduction, and in lower levels of total IgM JEV-specific neutralizing antibodies in mice models [39]. Burrack et al., also suggests that MDSC has an important suppressive T cells activity and may contribute to reduce the immune-mediated disease during Chikungunya infection [90].

Otherwise, the immunosuppressive activity triggered by RNA viruses, MDSC has been associated with metabolic regulation of immunopathology induced by DNA viruses, like hepatitis B virus (HBV) [91]. Pallett et al., 2015 showed that frequencies of MDSC on liver from HBV patients without liver damage, monitored by levels of liver transaminase enzymes, were higher in comparison with patients with liver damage, showing a protective effect for patients with immune-mediated viral disease, as hepatitis B [91].

In the new coronavirus pandemic (COVID-19), the MDSC have been reported to play an important role in the early phase of symptoms, increasing their frequency on blood in the first days of signals and symptoms, and it was related to poor outcome in severe acute respiratory syndrome in hospitalized patients. Pregnancy is a risk factor for COVID-19 severity, given the Brazilian high mortality rate of 12.7% in June 2020 withing pregnant, which may be associated with the change of the immunity [92–94].

Although few studies involving MDSC frequencies on blood during Zika infection were published yet, those cell type needs to be investigated, even though in animal models for medical science breakthroughs. The technique to characterize this cell phenotype is simpler than to characterize regulatory T cells, once the procedure does not require intracellular staining [95].

If those MDSC are crucial to maintaining a healthy pregnancy, any adverse effects, as Zika virus infection could trigger an imbalance between MDSC and T cells. This dysfunction may induce a deactivation of functional MDSC on blood and placenta with failure to attempt to eliminate viral infection. In addition, T cell function during ZIKV infection is known to be delayed throughout interferences

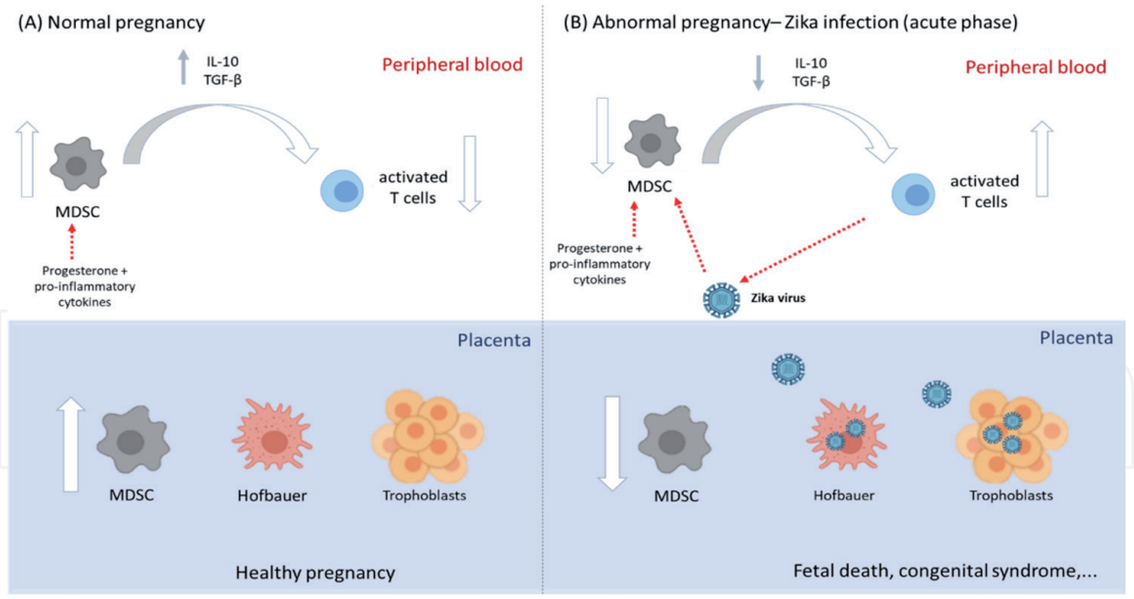

Figure 2.
Myeloid-derived suppressor cell (MDSC) activation and regulation triggered by normal pregnancy and by Zika virus infection. Summary of MDSC functionality during normal pregnancy (A) and during acute phase of Zika virus infection (B) as suggested by others into an innate immunity dysregulation observed in abnormal pregnancies on monkeys [35, 37, 38, 73, 77–81, 85]. Hormone and cytokines produced in normal pregnancy induce an equilibrium in peripheral blood maintaining frequency of MDSC elevated (1.A), as well as levels of IL-10 and TGF-beta. Meanwhile, circulating levels of T cell frequencies are reduced and controlled. In placenta, Hofbauer cells (macrophages) are responsible for immune surveillance also intermediating the cross-talking between fetus-maternal interface, with equilibrium of MDSC and T cells to maintain a healthy pregnancy. In abnormal pregnancy, also suggestive for Zika virus infection during pregnancy of non-human primates, the equilibrium is broken. Once ZIKV is circulating, there is a reduction of MDSC frequency (B), compromising pregnancy immunosuppression, with elevation of activated T cells, attempting to virus elimination. In the placental parenchyma, MDSC has a reduction in their frequency. This scenario also suggests an immune dysfunction in fetus-maternal environment, diminishing functional macrophages (Hofbauer cells), which are infected by virus. All events together can induce several poor outcomes (abortion, neurological disorders). Black arrows filled with white color represent the frequency of cells (up = high, down = low). Grey arrows represent levels of cytokines (up = high, down = low). Red dashed arrows represent the direction of function/induction events that have been known during Zika infection during pregnancy. Figure was created using Biorender software (https://www.biorender.com).

on interferon pathway, as described above. Then, this scenario may contribute to immune evasion of ZIKV, in which viral replication on maternal-fetal environment is unavoidable, inducing poor outcomes during pregnancy: fetal death, congenital syndrome, abortion, neurological disorders, etc. (**Figure 2**).

4. Programmed cell death: A host innate immune protection or a virus evasion strategy

It has been described that a protective response by innate immune cells to viruses is triggered by several distinct mechanisms including apoptosis, necrosis, paraptosis, pyroptosis, autophagy cell death, and others. Each one is depending on several aspects of infection, including where the microorganism was detected, susceptible target-cells, through signaling systems discharging the death signal, and its intensity. During the innate immune response to infections, programmed cell death may occur as a direct pathogenic mechanism of viral spread and escape from the immune system or represents an appropriate host response to limit pathogen replication. Apoptosis of lymphocytes and monocytes also plays an important role in the control of inflammatory responses, as well as in the development of maternal-fetal tolerance [96–99].

Type 1 programmed cell death, also known as apoptosis, is defined by internucleosomal DNA fragmentation, marked irreversible apoptotic characteristic indicating chromatin condensation, degradation of cytoskeleton and nuclear proteins, protein crosslinking, apoptotic bodies' formation baring ligands for receptors of phagocytic cells and, finally, the uptake by these phagocytes [97–99]. Type 2, or autophagic cell death, presents unique characteristics organelles formation including autophagosomes and autophagolysosomes in the dying cell, sources of self-degradation, and recycling [100].

Two pathways can regulate the apoptosis program in different aspects: extrinsic and intrinsic. Extrinsic pathway is activated by a transduction signal through death receptors, in which TNF, Fas ligand, or TRAIL bind to their respective receptors, such as TNF receptor family: TNFR1, Fas (CD95/APO-1) and TRAIL-R1/2. A complex signal mediated by this binding leads to an enzymatic cascade of cell degradation, and at this point caspase-3 is activated promoting DNA damage [101]. Intrinsic pathway involves intracellular mitochondria, which its membrane is the local for many Bcl-2 family members and their activity in inducing / inhibiting the mitochondrial apoptosis program implies in those proteins lead to membrane collapse as well as a transition from mitochondrial permeability promoting apoptosis process [96, 101–105].

Taking together, type 2, or autophagic cell death, consists of a conserved catabolic process that contributes to degradation and recycling of many intracellular substances, through lysosome activity. In this sense, many studies have shown its importance in immune responses, including degradation of microbes, direct viral peptides MHC class I presentation [106] and even altering T-cell signaling and tolerance [107, 108]. At first, autophagy is necessary to keep the cell alive under stress conditions that precede their demise. Such kind of cell death could be achieved by several mechanisms, including prolonged hypoxia or digestion of vital factors, regulatory molecules or essential organelles. In a stress situation, caused by virus, an infected cell can induce intracellular signals of autophagy, inhibiting cell proliferation, arresting cell cycle and eventually leading to cell death [106–111].

In the acute ZIKV infection during pregnancy, macrophages and dendritic cells are involved in inflammatory cytokines production, in which CARD9 expression, an important regulator of caspase activity playing an important role in cell apoptosis regulation, is elevated allowing that pattern recognition receptors (PRR) induce pro-inflammatory cytokines cascade, as the first step on CZS, as suggested [67]. According to Quicke et al., Hofbauer cells infected with ZIKV in placenta induces IFN type I activation, reactive oxygen species production, as well as pro-inflammatory cytokines, but with minimal cell death, showing a scape of innate immune response [23]. Recently, Cao et al., showed that ZIKV could activate and increase an autophagic process in pregnant mice, suggesting an imbalance of trophoblastic cells in placenta, and relation with fetal loss [112]. Corroborating, Ribeiro et al. using a human model of placenta explants for in vitro infection demonstrated tissue injury as consequence of the association between fetal pro-inflammatory responses mediated by IL-1β, IL-6 and TNF and extrinsic caspase 3 dependent apoptosis (TNF-TNFR pathway). Together data suggest that ZIKV infection corroborates to placenta innate immune and hormonal dysfunction, increasing loss barrier integrity [42] Thus, this inflammatory status could trigger cell death and barrier loss, allowing ZIKV cross placenta and infect fetuses' neural stem cells (**Figure 3**) [23, 113–115]. Interesting, autophagosomes are present in neural stem cells and it could facilitate ZIKV replication [116], although inflammation generated as well as the cytopathic effect itself culminate in extensive caspase-dependent neuronal cell death.

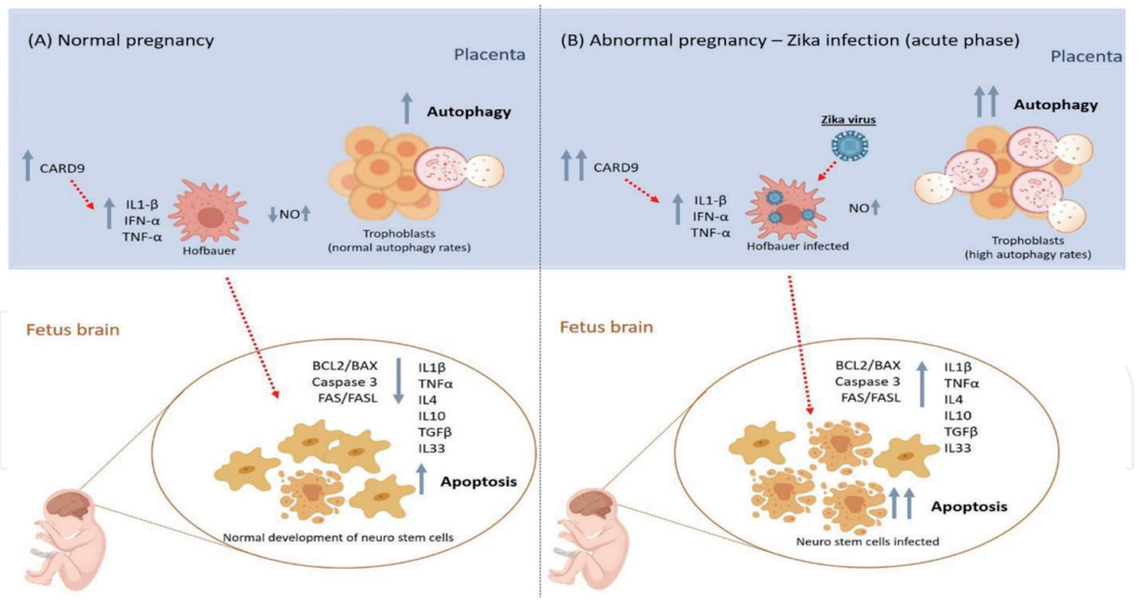

Figure 3.
Programmed cell death activation during normal pregnancy and abnormal pregnancy induced by Zika virus. Normal pregnancy equilibrium is driven by regulation of number of innate immune cells in placenta leading by programmed cell death. In this situation, caspase activity starts on CARD9 expression with cytokines production by Hofbauer cells (1.A), which oxide nitric (NO) regulates trophoblasts autophagy (2.A, 3.A). Products of Hofbauer cells activity in the surveillance in placental parenchyma contributing to extrinsic (Fas/Fas-L) and intrinsic pathway (BCL2/BAX) activation in fetus brain with low expression of pro-inflammatory cytokines, regulating number of neural stem cells and microglia by apoptosis (4.A), maintaining the healthy pregnancy. Acute ZIKV infection during pregnancy suggests that macrophages and DCs are involved in pro-inflammatory cytokines production, in which CARD9 is upregulated, increasing caspase activity, allowing pro-inflammatory cytokines and reactive species cascade (1.B, 2.B), exacerbating autophagy in placenta (3.B). Taking together this innate immune dysfunction, fetus brain is affected by high activation of apoptosis pathway (4.B), provoking a cascade of cell death with an abrupt reduction of neural cells, causing severe damage [113–115]. Grey arrows represent the production or expression levels (up = high, down = low). Double arrows represent a high magnitude of production or expression. Red dashed arrows represent the direction of function/induction events that have been known and those suggested. Figure created using Biorender software (https://www.biorender.com).

Corroborating, Lum et al. has shown that ZIKV mainly infects fetal microglia and induces high levels of pro-inflammatory cytokines that could be harmful to the fetus [117]. In addition, the analysis of in vitro culture, fetal brain histology and *ex vivo* studies with children presenting evidence of congenital infections demonstrated that, in fact, ZIKV promotes microglial activation, suggesting viral disseminating, neuronal death and an abnormal increase of astrocytes due to neurons destruction [117].

Thus, once in fetus central nervous system, ZIKV may contribute to extrinsic (Fas/Fas-L) and intrinsic (Bcl-2) pathways activation for programmed cell death, reducing number of neuronal cells. Thus, the risk of congenital syndrome is eminent, mainly in the first trimester, as well documented (**Figure 3**) [67, 118–123]. Some studies with fetuses' autopsies and infants with microcephaly have been demonstrated a broad spectrum of microscopic neuropathological abnormalities and brain damage, with direct virus cytopathic effects in neural glial cells. In this way, these data support the strong association with apoptotic cell death and micro-calcifications [13, 23, 124].

5. Prevention and control of ZIKV infection: Potential candidates in pregnant women

In general, pregnancy is a challenge for prevention and control infectious diseases regard to a safe drug or vaccine development to do not disturb the innate/adaptive

immunity homeostasis, however, there were no drugs approved for ZIKV infection treatment [28–30]. Here, drugs and vaccines candidates tested in animal models or in newborns will be described with details (**Table 1**).

5.1 Type III interferon: Potential efficacy and safety for immunotherapy

Type III interferon has been emerging as an efficient and low damaging therapeutic agent not only directed for the virus but also for fungal and bacterial infections, as well as cancer, autoimmune, and vascular diseases [54]. The more restricted expression of IFNLR1 likely contributes to the improved safety profile of IFN-λl in clinical studies compared to type I IFN. Pegylated IFN-λ1 have already been tested in phase 2b clinical trial to chronic hepatitis C treatment and hepatitis B, associated with improved rates of virologic response with fewer extrahepatic adverse events compared to pegylated IFN-α [125]. Even though it was deemed less effective than alternative treatments for these infections, pegylated- IFN- λ can be potential candidate ready for deployment if new indications are identified [126]. There are other viral targets for IFN- λ therapy been tested in murine models: norovirus [127], and influenza virus [128], and west nile virus – last one is another member of Flaviviridae family. It is noteworthy the effect of IFN-λ on infection with west nile virus, an encephalitic flavivirus: Treatment of IFNLR1 knockout mice with pegylated IFN-λ2 resulted in decreased blood–brain barrier permeability, reducing west nile virus infection in the brain without affecting viremia, and improved survival against lethal virus challenge [129].

The effectiveness and low damage treatments for other correlated viral infections, combined with the protagonist of IFN-λs as immunoregulatory and antiviral agent in ZIKV raise the idea of IFN-λs as ZIKV therapy, and some groups already achieve exciting good results. Concerning ZIKV infections, Jagger, et al., (2017) suggest that IFN-λ2 treatment could be a safe solution to minimize Congenital Zika Syndrome severe outcomes. Using a type III interferon-deficient mouse model, authors showed that these animals had an increase of ZIKV replication in the placenta under ZIKV infection, and treatment of pregnant mice with IFN-λ2 reduced ZIKV viremia [26]. Considering the vaginal epithelium as the first line of defense against sexually transmitted ZIKV, treatment of primary human vaginal and cervical epithelial cells lineages with IFN-λ induces host defense transcriptional signatures with augmented expression of ISGs (IFI44L, OASL, OAS1, and MX1) and inhibition of ZIKV replication. Female mice submitted to treatment with IFN-λ and intravaginal ZIKV transmission showed low levels of virus replication in the female reproductive tract with a hormonal stage-dependent role [130].

5.2 Direct-acting antiviral therapy based on RNA-dependent RNA polymerase inhibitors

Some studies were driving to evaluate effects of independent direct-acting antiviral drugs on Zika virus infection (**Table 1**), as sofosbuvir, an FDA-approved nucleotide analog inhibitor of the hepatitis C (HCV) RNA-dependent RNA polymerase (RdRp) [131, 132]. In vitro and *in vivo* studies have been demonstrated effectiveness of sofosbuvir as antiviral drugs to treat Zika and Dengue virus infection [133–135]. Mesci et al., 2018 reported that sofosbuvir was promisor to block vertical transmission of Zika virus in pregnancy using mice models [136]. Again, sofosbuvir shows to play a role in virus replication inhibition. Another flaviviral inhibitor NITD008, an adenosine analog inhibiting the RNA-dependent RNA polymerase activity through chain-termination [137], has been shown to reduce the

Therapy	classification	Mechanism of action	Immune effect	Pregnancy safety	References
Peg Interferon-λ2	Not approved	Antiviral immunobiological	Enhance IFNL-λ pathway activity	Yes/Mice models	Jagger et al., 2017 [26]
Sofosbuvir	Category B/Approved for hepatitis C treatment	Direct-acting antiviral drugs	Not explored	Yes/Mice models	Mesci et al., 2018 [136]
NITD008	Not approved	Direct-acting antiviral drugs	Not explored	Yes/Mice models	Watanabe et al., 2019 [27]
Hydroxycloroquine	Category C/Approved for malaria and autoimmune diseases therapy	Cell membrane interaction to induce cell death	Reduction of autophagy activity	Yes/Pregnant women	Cao et al., 2017 [112]
rVSV vaccine	Not approved	Recombinant viral vector vaccine	Increases in CD8+/CD44high/IFN-γ + T cell populations on spleen	Yes/Mice models	Betancourt et al., 2017 [147]
VRC5283	Clinical trial phase II (VRC-ZKADNA090–00-VP)	DNA plasmid vaccine	Induce antigen-specific antibody production/ induce of CD8+ T cells response	Yes/Mice models	Richner et al.,2017 [155]
mRNA-LNP vaccine	Clinical trial phase I (NCT03014089)	mRNA vaccine	Induce antigen-specific antibody production/ induce of CD8+ T cells response/Minimizes ADE	Yes/Mice models	Richner et al.,2017 [156]

Table 1.
Therapeutic agents or vaccine candidates targeting virus or immunity with promisor potential to use during ZIKV infection in pregnant women.

Zika virus replication in placenta, and fetal infection, thus minimizing the risk of maternal-fetal transmission of ZIKV [27].

There are few studies investigating innate immunity during antiviral therapy, especially when its concern to Flaviviridae family [38, 135, 138, 139]. Scarce literature revealed knowledge about antiviral therapy immune effects only during hepatitis C infection [138, 139]. Antiviral drugs, as pegylated interferon (PEG-IFN), ribavirin, and direct-acting antiviral agents (DAA) have been related with a reduction of innate regulatory cells, as MDSC, in peripheral blood from hepatitis C chronic patients, in which T cells were increased and immune function was reestablished [138, 139]. Nevertheless, all those drugs are aimed to interrupt viral replication and any dysregulation of immune cells during pregnancy is not safe, then those drugs are not recommended to be used during gestational period [140]. Besides no immune response evaluation was related to DAA therapy, it has been known that small molecules with specific activity should not induce any immune alterations in maternal-fetal immunity [140].

Safety and effectiveness of sofosbuvir on Zika virus infection should be addressed to immune response evaluation, which is poorly explored, even more in pregnant animal models. More studies and investments are needed for non-clinical and clinical studies, to get safety therapeutic protocols aimed to pregnant women with Zika virus or other flavivirus infection.

5.3 Cell death modulation during antiviral therapy

Genetic manipulation has been proven to be a promising tool for vaccine and therapy development. Considering the type 2 of programmed death, autophagy is activated by ZIKV in placental parenchyma and is involved in poor outcome during pregnancy, this cell death pathway has been a target for therapies [112, 141–143].

Recently, a study showed the role of an autophagy gene (Atg16I1) during ZIKV infection in pregnant mice model, in which inducing a deficiency in this gene limited ZIKV vertical transmission, as well fetal damage, improving placental and fetal outcomes [112]. In addition, an antiviral compound approved to be used by pregnant women for malaria and autoimmune diseases [141], hydroxychloroquine (HCQ), has been used to dampen autophagic activity *in vivo* [142]. Thus, Cao et al., showed that HCQ administered with a dose of 40 mg/kg/day has *in vivo* inhibitory effects on autophagy sustained lower levels of ZIKV RNA compared with saline buffer treatment [112].

Based on the knowledge of ZIKV infection that can trigger a caspase-3 activation contributing to cell death of neural progenitor cells during pregnancy, it is an extremely relevant approaches targeting cell death pathways for antiviral treatments even though for therapeutic vaccines.

5.4 Recombinant viral vectors as vaccine candidate

Recombinant viral vectors have been highlighted as therapeutic alternatives to prevent and treat infectious disease [144, 145], considering its specificity and the adverse effects of antiviral drugs and some vaccines [140, 146]. Betancourt et al., 2017 showed that a recombinant viral vector from vesicular stomatitis virus (rVSV) anti-ZIKV vaccine increased IFN-γ production by splenic CD8+ T cells as well as high neutralizing anti-ZIKV antibody titers from pregnant mice. This study also demonstrates that neonatal mouse from vaccinated dams was partially protected against neurological manifestations of ZIKV infection following wild-type virus challenge [147]. This rVSV using pre membrane and envelope region together obtained from a ZIKV strain as reference had the potential to protect from ZIKV

infection during prenatal and neonatal development, likely through the transmission of maternal IgG. Despite rVSV vaccine induces IFN-γ production in pregnant mice, this vaccine needs to be evaluated for other types of interferon, mainly its effects on placental tissues .

5.5 Potential DNA and mRNA vaccines

mRNA vaccines as well as DNA-based vaccines represent a versatile vaccine platform and an alternative to conventional vaccine approaches because of their high potency, capacity for rapid development and potential for low-cost manufacture and safe administration [148]. Recent technological advances have allowed mRNA vaccines to demonstrate encouraging results in both animal and human models. Regarding prophylactic mRNA vaccines, a number of reports have demonstrated the potency and versatility of mRNA to elicited protective immunity against a variety of infectious agents in animal models against, including influenza virus, Ebola virus, Zika virus, Human Immunodeficiency virus 1 (HIV-1), herpes simplex virus, cytomegalovirus, hepatitis C and respiratory syncytial virus [149–151]. It has been noted that approximately ten mRNA vaccines programs have entered clinical trials [152].

The importance of mRNA-based vaccines and therapies is emphasized when mRNA-based biopharmaceuticals are entering the market with guidance of new biopharmaceutical companies. Modern Therapeutics, an mRNA therapy company evaluated various mRNA vaccine technologies to identify immunogenic and scalable candidates. The pipeline of this company shows different investigative stages mRNA vaccines of the following vaccines Respiratory Syncytial virus (RSV), Cytomegalovirus (CMV), human metapneumovirus (hMPV) + Parainfluenza virus Type 3 (PIV3), Influenza A subtypes H10N8, and H7N9, Zika, and Chikungunya. Curevac is the first biopharmaceutical company that developed the first prophylactic mRNA vaccine in the clinics, recently they showed that RNActive® vaccines induced long-lived and protective immunity to influenza A virus infections in various animal models [153].

Thus, big pharmaceutic companies, such as Merck & Co., have been invested in Modern Therapeutics aiming to expand the field of mRNA vaccine (https://www.modernatx.com/). Indeed, nucleic acid vaccine platform has been presented to combat the emergence of acute viral diseases, mainly to rapidly contain emerging outbreaks before they spread out of control. In this context, two vaccines were developed to combat the ZIKV outbreak (1) DNA plasmid vaccine encoding the prM-E genes of ZIKV and (VRC5283) (2) mRNA vaccine (mRNA-LNP), both vaccines mediate protection from ZIKV infection in mouse models. The DNA plasmid vaccine is in phase 2 human clinical trials (VRC-ZKADNA090–00-VP) and vaccine mRNA-LNP is in phase 1 clinical trial (NCT03014089) [154–156].

Considering that vaccine trials might not be performed in pregnant women and have not yet tested vaccines against ZIKV vertical transmission, there is a need for establishing the efficacy of ZIKV vaccines against mother-to-child transmission in animal models. In order to address those questions, it has been shown that vaccination with DNA plasmid encoding Zika virus prM-E and a lipid-encapsulated mRNA vaccine-elicited antigen-specific antibody and CD8+ T cell responses in mice, being able to generate a high level of protection against vertical transmission. Moreover, the mRNA-LNP vaccine not only inhibited vertical transmission but also ensured that fetuses are protected therefore, reinforcing its potential as promising vaccine for pregnant women [155]. Since there are few studies in the field of ZIKV vaccine candidates that evaluated vertical transmission, intrinsic maternal factors as well as fetal health, nucleic acid vaccines are pointed as a great opportunity to contain ZIKV infection.

6. Conclusion

Considering the normal pregnancy, the innate immunity balance is conduct by downregulation of effector T cells and NK cells leading by innate regulatory cells (MDSC) and upregulation of pro-inflammatory cytokines. This innate immune modulation that occurs mainly at the placenta, includes interferon pathway and cell death modulation as shown in **Figure 4A**. Gestation has its own difficulties to successful outcomes regarding maternal immune tolerance. Zika virus infection becomes classified as disease-causing birth defects, developing an abnormal pregnancy, as consequence of immune dysregulation (**Figure 4B**). Thus, antiviral therapy is the key to control this immune imbalance showing positive effects in innate immunity on pregnant mice models. It has been known that efforts through vaccines development targeting pregnant women will be the solution for ZIKV prevention, as well as for other arboviral infections, to maintain immune homeostasis and generate healthy babies. Finally, this chapter brings some new thoughts that help for targeted improvements in medical science considering Zika infection

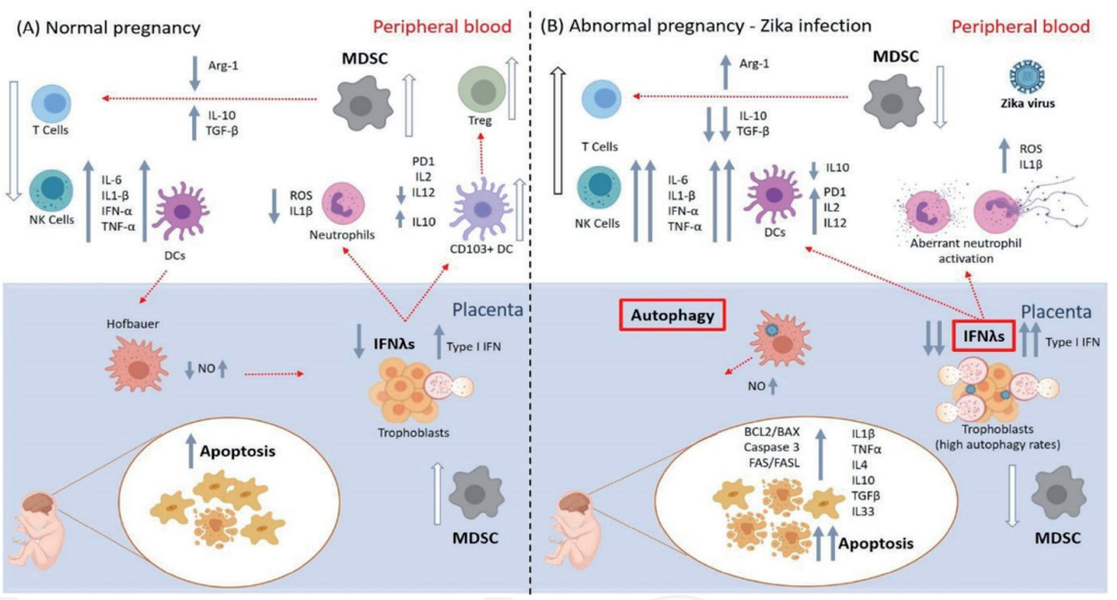

Figure 4.
Summary of innate immunity functionality during normal pregnancy and in Zika virus infection focus on interferon III, myeloid-derived suppressor cells, and programmed cell death activities. During pregnancy, initial signal is dependent on nidation process and placenta formation leading by trophoblasts expansion and activation. Following this process, innate cells, such as neutrophils, DCs, and cytokines are activated (1.A, 2.A) with IL10 and TGF-beta production in periphery, allowing immunosuppressive functionality triggered by regulatory cells (MDSC and Treg) (3.A). This condition facilitates suppression of effector cells (NK and lymphocytes) in peripheral blood and in placenta triggered by MDSC (4.A), whereas Hofbauer cells maintain reactive species (NO) balanced (5.A) as well as the IFN-λ downregulation, IFN type I upregulation, and trophoblast autophagy (6.A), contributing to the cross-linking in the fetus-maternal interface. Adding to that, programmed cell death contributes to control the accelerated growth of neural cells in fetus brain (7.A), corroborating with a successful pregnancy. Zika virus has been related to abnormal pregnancy, leading to massive innate immune alteration, causing severe brain damage to fetus. Given that, when the virus is in the blood, there is a gross activation of innate cells, elevation of cytokines and chemokines (1.B, 2.B), and suppressive activity by regulatory cells is compromised (3.B), generating early activation of NK and T cells in blood (4.B) and macrophages in placenta (5.B). Virus invasion in placenta through Hofbauer and trophoblast cells results in high autophagy activity with interferon type I gene highly expressed combined with super downregulation of interferon type III (6.B). This imbalance also contributes to fetal brain damage, orchestra by high activation of apoptosis pathway, avoiding neural cells growing progress. Thus, Zika provides severe damage to fetus, in which drugs, vaccines and immunotherapies have been designed suggesting a modulation of three important keys of innate immunity to control virus replication and spread into fetus-maternal interface: interferon type III expression, MDSC frequency, and autophagy process (highlighted with red rectangles) to avoid severe fetus brain damage, allowing a healthy pregnancy. This figure was made based on the information from ***Figures 1–3****. Figure created using Biorender software (https://www.biorender.com).*

on pregnancy, and innate immune system linked to therapies previewing the prevention and control.

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Conflict of interest

Authors to declare no conflicts of interest.

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