

## ANTIVIRAL IMMUNITY

# Antiviral immunity backfires: Pathogenic effects of type I interferon signaling in fetal development

Rebecca L. Casazza and Helen M. Lazear\*

Interferon- $\alpha/\beta$  signaling is pathogenic in a mouse model of congenital Zika virus infection.

Pregnancy is an immunological balancing act; maternal and fetal immunity act in concert to protect the fetus from maternal pathogens while preventing maternal immune attack of the semiallogeneic fetus and placenta. Few pathogens are able to circumvent the specialized immune mechanisms that protect the maternal-fetal interface (1). The most recently recognized congenital pathogen is Zika virus (ZIKV), a mosquito-borne flavivirus that can produce a spectrum of neurodevelopmental defects (congenital Zika syndrome). In addition to vision and hearing loss, seizures, and microcephaly, congenital ZIKV infection is also associated with placental damage, leading to intrauterine growth restriction (IUGR) and fetal demise. The ability of ZIKV to cause birth defects was unexpected because this is not characteristic of related flaviviruses and had not been reported in ZIKV outbreaks before 2015. Understanding the mechanisms that allow ZIKV to breach the placental barrier and damage the developing fetus is important for combating ZIKV and other congenital infections.

In this issue of *Science Immunology*, Yockey *et al.* demonstrate that type I interferon (IFN- $\alpha/\beta$ ) signaling is pathogenic in a mouse model of congenital ZIKV infection, resulting in abnormal placental architecture, IUGR, and fetal demise (Fig. 1) (2). This finding is surprising because IFN- $\alpha/\beta$  induces a potent antiviral response, and mice that lack the IFN- $\alpha/\beta$  receptor (*Ifnar1*<sup>-/-</sup>) are generally more susceptible to viral infections. ZIKV replicates poorly in mice because it does not efficiently antagonize mouse IFN- $\alpha/\beta$  signaling, so existing ZIKV pathogenesis models use *Ifnar1*<sup>-/-</sup> mice or an IFNAR1-blocking monoclonal antibody to achieve sufficient viremia (3, 4). *Ifnar1*<sup>-/-</sup> mice have been used to demonstrate transplacental ZIKV infection and subsequent fetal damage after subcutaneous or intravaginal infection (3, 5, 6). In these models, *Ifnar1*<sup>-/-</sup> dams were crossed to wild-type sires, resulting in *Ifnar1*<sup>+/-</sup> fetuses. A strength of these systems is that the fetus

(and thus placenta) have intact IFN- $\alpha/\beta$  signaling, so ZIKV infection of the placenta and fetus is not simply due to a lack of an innate antiviral response in these tissues. The present discovery by Yockey *et al.* results from a different mating strategy: Rather than crossing *Ifnar1*<sup>-/-</sup> dams to wild-type sires, they used *Ifnar1*<sup>+/-</sup> sires, resulting in pregnancies in which 50% of the fetuses (and their associated placentas) were *Ifnar1*<sup>+/-</sup>, whereas 50% were *Ifnar1*<sup>-/-</sup>. Unexpectedly, they found fetal demise exclusively in *Ifnar1*<sup>+/-</sup> fetuses, which also exhibited greater IUGR compared with *Ifnar1*<sup>-/-</sup> fetuses, suggesting that IFN- $\alpha/\beta$  signaling was pathogenic rather than protective. Consistent with previous reports (3, 6), the outcome of congenital ZIKV infection depended on gestational stage, with infection at embryonic day 5.5 (E5.5) resulting in fetal demise (resorption), whereas infection at E8.5 resulted in IUGR. Although *Ifnar1*<sup>-/-</sup> fetuses exhibited less severe pathology compared with that of *Ifnar1*<sup>+/-</sup> littermates, ZIKV replicated to higher titers in *Ifnar1*<sup>-/-</sup> placentas, indicating that IFN- $\alpha/\beta$  restricts ZIKV infection in the placenta and identifying a pathogenic role for IFN- $\alpha/\beta$  independent of viral replication.

Because all ZIKV infections were performed in *Ifnar1*<sup>-/-</sup> dams, only placental and fetal tissues could respond to IFN- $\alpha/\beta$  in this system. Accordingly, the authors observed substantial malformation in *Ifnar1*<sup>+/-</sup> placentas after ZIKV infection and minimal damage in *Ifnar1*<sup>-/-</sup> placentas, even though *Ifnar1*<sup>-/-</sup> placentas exhibited equivalent or higher levels of viral infection. In both mouse and human placentas, fused syncytiotrophoblasts (a single layer in human and double layer in mouse) form a critical barrier that separates the maternal and fetal blood supplies and restricts hematogenous pathogen transmission. Electron microscopy of *Ifnar1*<sup>+/-</sup> placentas after ZIKV infection revealed disrupted syncytiotrophoblast barriers and consequent mixing of maternal and fetal blood. To determine the mechanism by which IFN- $\alpha/\beta$

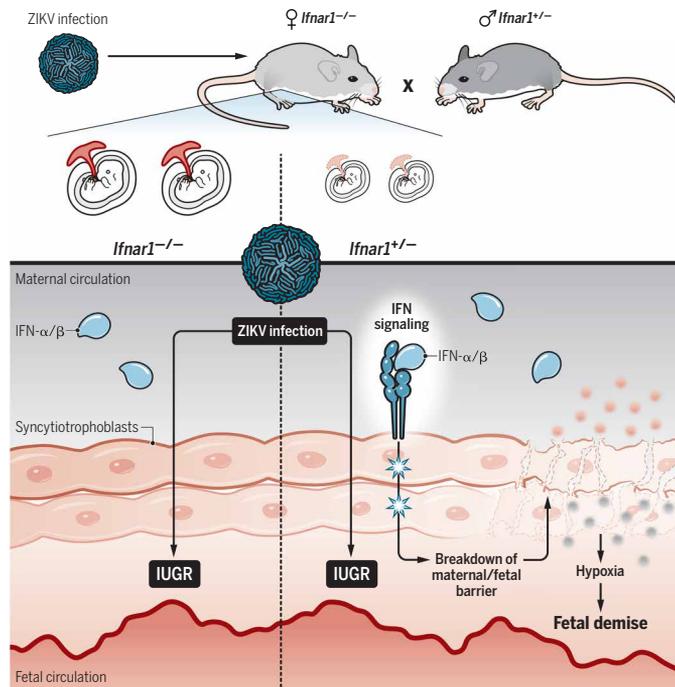
signaling disrupted placental architecture, the authors considered whether IFN- $\alpha/\beta$  recruited immune cells to the placenta, blocked placental cell proliferation, or induced apoptosis after ZIKV infection. They found little leukocyte invasion at the maternal-fetal interface and no difference between *Ifnar1*<sup>+/-</sup> and *Ifnar1*<sup>-/-</sup> placentas. Likewise, *Ifnar1*<sup>+/-</sup> and *Ifnar1*<sup>-/-</sup> placentas showed no difference in the expression of the proliferation marker Ki67. In contrast, cleaved caspase-3 was detected only in *Ifnar1*<sup>+/-</sup> placentas, suggesting that IFN- $\alpha/\beta$  disrupts placental integrity by inducing trophoblast apoptosis. To test whether IFN- $\alpha/\beta$  signaling results in placental insufficiency, contributing to IUGR and fetal demise, the authors compared the expression of hypoxia-induced genes in *Ifnar1*<sup>+/-</sup> and *Ifnar1*<sup>-/-</sup> fetuses. All hypoxia response genes tested, including *Vegfa* and *Adm*, were up-regulated in *Ifnar1*<sup>+/-</sup> fetuses after ZIKV infection. Moreover, hypoxia response transcripts were up-regulated at developmental stages closely preceding demise, suggesting that nutrient and oxygen insufficiency contribute to the loss of *Ifnar1*<sup>+/-</sup> fetuses.

To determine whether IFN- $\alpha/\beta$  signaling is also pathogenic in the human placenta, Yockey *et al.* assessed the effects of IFN treatment on explants of human midgestation placental villi. They found that exogenous IFN- $\beta$  treatment altered the villus architecture, with the trophoblast actin cytoskeleton becoming disrupted and areas of aggregated nuclei forming at villus tips. Similar knot or sprout structures are seen in other placental pathologies, so the presence of these aggregates is consistent with IFN- $\alpha/\beta$  causing structural damage to the placenta. Placental deformities were only observed after treatment with IFN- $\beta$  and not with IFN- $\lambda$  (type III IFN), which signals through a different receptor. Human syncytiotrophoblasts produce high levels of IFN- $\lambda$  *ex vivo*, and IFN- $\lambda$  restricts ZIKV transplacental transmission in mice, so IFN- $\lambda$  has been suggested to play an important role in restricting congenital infections (6, 7). RNA sequencing analysis revealed that IFN- $\beta$  and IFN- $\lambda$  induced

Copyright © 2018  
The Authors, some  
rights reserved;  
exclusive licensee  
American Association  
for the Advancement  
of Science. No claim  
to original U.S.  
Government Works

Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA.  
\*Corresponding author. Email: helen.lazear@med.unc.edu

**Fig. 1. IFN- $\alpha/\beta$  signaling exacerbates adverse pregnancy outcomes following ZIKV infection.** *Ifnar1*<sup>-/-</sup> fetuses exhibit more pathology than their *Ifnar1*<sup>+/-</sup> littermates after maternal ZIKV infection. ZIKV infection itself causes damage in *Ifnar1*<sup>-/-</sup> placentas and fetuses (evident as IUGR in *Ifnar1*<sup>-/-</sup> fetuses). However, IFN- $\alpha/\beta$  signaling induces additional pathogenic effects in *Ifnar1*<sup>+/-</sup> fetuses, including more severe IUGR, hypoxia, placental damage, and fetal demise.



distinct transcriptional signatures in placental explants, which is surprising because IFN- $\beta$  and IFN- $\lambda$  are generally thought to activate similar transcriptional programs. Together, these observations support a model in which IFN- $\lambda$  elicits a distinct antiviral response at the maternal-fetal interface, perhaps to avoid the pathologic effects of IFN- $\alpha/\beta$  signaling.

Remarkably, the authors found that IFN- $\alpha/\beta$  was pathogenic even in the absence of viral infection: Wild-type dams exhibited fetal demise when treated with polyinosinic:polycytidylic acid [poly(I:C)], a double-stranded RNA mimic that induces robust IFN- $\alpha/\beta$  production. *Ifnar1*<sup>+/-</sup> fetuses were resistant to the effects of poly(I:C) in *Ifnar1*<sup>-/-</sup> dams, indicating that IFN- $\alpha/\beta$  signaling in maternal tissues triggered fetal demise. In the context of ZIKV infection, IFN- $\alpha/\beta$  signaling in the placenta mediated tissue damage, but the IFN- $\alpha/\beta$  itself could derive from the maternal circulation or from the infected fetal/placental tissue. In addition, *Ifnar1*<sup>-/-</sup> mice sustain very high levels of IFN- $\alpha/\beta$ , owing to uncontrolled viral replication and a lack of receptor to sequester IFN from the circulation. Because the disruptive effects of IFN- $\beta$  in human placental explants were induced by high doses of IFN- $\beta$ , it remains to be determined whether lower levels of IFN- $\alpha/\beta$  can also cause placental pathology.

Although sufficient to cause fetal demise after poly(I:C) administration, maternal IFN- $\alpha/\beta$

signaling alone is unlikely to explain the ability of ZIKV to cause neurodevelopmental defects. Nearly all viral infections induce IFN- $\alpha/\beta$  production, and there is no reason to think that ZIKV induces an especially robust IFN response. Dengue virus (DENV), a mosquito-borne flavivirus closely related to ZIKV, typically causes a more severe febrile illness than does ZIKV, and secondary DENV infections can result in cytokine storm and shock. DENV infection during pregnancy is associated with adverse fetal outcomes, likely related to the maternal antiviral response (8). Nonetheless, despite millions of DENV infections worldwide annually, there is no evidence that DENV causes birth defects of the sort caused by ZIKV. Thus, IFN- $\alpha/\beta$  signaling may cause placental damage that contributes to ZIKV pathogenesis, but additional features of ZIKV (perhaps including neurotropism) must contribute to this virus's ability to cause birth defects. However, the detrimental effects of IFN- $\alpha/\beta$  signaling could also be implicated in poor pregnancy outcomes during other viral infections. In one model, maternal IFN- $\alpha/\beta$  production might induce placental damage, facilitating hematogenous viral invasion into the fetal compartment; in this case, many maternal pathogens might cause placental damage, although only a subset have the ability to productively infect the placenta and fetus. Alternatively, pathologic IFN- $\alpha/\beta$  signaling might occur subse-

quent to placental infection; in which case, only the few pathogens already able to breach the placental barrier could elicit a damaging IFN- $\alpha/\beta$  response.

Pathogens can access the fetal compartment via two routes: hematogenous transplacental infection or ascending transvaginal infection, with each route comprising distinct anatomical and immunological barriers. ZIKV is transmitted primarily by mosquitoes; however, one unusual feature of ZIKV among flaviviruses is that ZIKV can also be sexually transmitted between humans. However, it remains unclear whether the route by which a pregnant woman is exposed to ZIKV influences the probability or outcome of congenital infection. ZIKV replicates in tissues of the female genital tract, so sexual transmission could result in ascending infection. Alternatively, sexual transmission could result in maternal viremia and transplacental infection indistinguishable from mosquito-borne transmission. Yockey *et al.* mainly used an intravaginal infection model for their studies. Their key finding—maternal ZIKV infection resulted in demise exclusively of *Ifnar1*<sup>+/-</sup> fetuses—was also observed after subcutaneous inoculation, indicating that this outcome is not exclusive to intravaginal inoculation. However, differences in experimental design (such as virus strain and timing of infection) mean that the importance of intravaginal infection compared with subcutaneous infection remains to be determined.

Pregnancy features dynamic and highly regulated inflammatory responses (9). Inflammatory cytokines promote implantation and later contribute to the onset of labor, both at term and in response to preterm labor triggers such as infection. However, most fetal development occurs during a period of suppressed inflammation, a key purpose of which is to prevent the maternal immune response from attacking the semiallogeneic fetus. This aspect of pregnancy is not present in congenic mouse models, including the C57BL/6 mice used in the present study and by other groups, because the mother and fetus are genetically identical (except for *Ifnar1* status). Immune responses to congenital infections such as ZIKV may disrupt the inflammatory balance of pregnancy, allowing the development of immune pathology in the placenta. Furthermore, autoimmune conditions characterized by excess IFN- $\alpha/\beta$  production, including Aicardi-Goutières syndrome and systemic lupus erythematosus (10), are associated with many of the same poor pregnancy outcomes seen with congenital infections. The work of Yockey *et al.* suggests

a general mechanism by which IFN- $\alpha/\beta$  signaling could contribute to placental pathology in a variety of pregnancy complications.

## REFERENCES

1. C. B. Coyne, H. M. Lazear, Zika virus—Reigniting the TORCH. *Nat. Rev. Microbiol.* **14**, 707–715 (2016).
2. L. J. Yockey, K. A. Jurado, N. Arora, A. Millet, T. Rakib, K. M. Milano, A. K. Hastings, E. Fikrig, Y. Kong, T. L. Horvath, S. Weatherbee, H. J. Kliman, C. B. Coyne, A. Iwasaki, Type I interferons instigate fetal demise after Zika virus infection. *Sci. Immunol.* **3**, eaao1680 (2017).
3. L. J. Yockey, L. Varela, T. Rakib, W. Khoury-Hanold, S. L. Fink, B. Stutz, K. Szigeti-Buck, A. Van den Pol, B. D. Lindenbach, T. L. Horvath, A. Iwasaki, Vaginal exposure to Zika virus during pregnancy leads to fetal brain infection. *Cell* **166**, 1247–1256.e4 (2016).
4. H. M. Lazear, J. Govero, A. M. Smith, D. J. Platt, E. Fernandez, J. J. Miner, M. S. Diamond, A mouse model of Zika virus pathogenesis. *Cell Host Microbe* **19**, 720–730 (2016).
5. J. J. Miner, B. Cao, J. Govero, A. M. Smith, E. Fernandez, O. H. Cabrera, C. Garber, M. Noll, R. S. Klein, K. K. Noguchi, I. U. Mysorekar, M. S. Diamond, Zika virus infection during pregnancy in mice causes placental damage and fetal demise. *Cell* **165**, 1081–1091 (2016).
6. B. W. Jagger, J. J. Miner, B. Cao, N. Arora, A. M. Smith, A. Kovacs, I. U. Mysorekar, C. B. Coyne, M. S. Diamond, Gestational stage and IFN- $\lambda$  signaling regulate ZIKV infection in utero. *Cell Host Microbe* **22**, 366–376.e3 (2017).
7. A. Bayer, N. J. Lennemann, Y. Ouyang, J. C. Bramley, S. Morosky, E. T. De Azeved Marques Jr., S. Cherry, Y. Sadovsky, C. B. Coyne, Type III interferons produced by human placental trophoblasts confer protection against Zika virus infection. *Cell Host Microbe* **19**, 705–712 (2016).
8. E. S. Paixão, M. G. Teixeira, M. d. C. N. Costa, L. C. Rodrigues, Dengue during pregnancy and adverse fetal outcomes: A systematic review and meta-analysis. *Lancet Infect. Dis.* **16**, 857–865 (2016).
9. G. Mor, P. Aldo, A. B. Alvero, The unique immunological and microbial aspects of pregnancy. *Nat. Rev. Immunol.* **17**, 469–482 (2017).
10. Y. J. Crow, N. Manel, Aicardi–Goutières syndrome and the type I interferonopathies. *Nat. Rev. Immunol.* **15**, 429–440 (2015).

10.1126/sciimmunol.aar3446

**Citation:** R. L. Casazza, H. M. Lazear, Antiviral immunity backfires: Pathogenic effects of type I interferon signaling in fetal development. *Sci. Immunol.* **3**, eaar3446 (2018).