The Journal of Immunology

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J Immunol 2017; 198:3029-3032; Prepublished online 6 March 2017;
doi: 10.4049/jimmunol.1601824
http://www.jimmunol.org/content/198/8/3029

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Cutting Edge

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Cutting Edge: Fetal/Placental Type I IFN Can Affect Maternal Survival and Fetal Viral Load during Viral Infection

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Pregnant women have greater mortality and complications associated with viral infections compared with the general population, but the reason for the increased susceptibility is not well defined. Placenta type I IFN is an important immune modulator and protects the pregnancy. We hypothesized that loss of placental IFN affects the regulation of the maternal immune system, resulting in the differential response to infections observed in pregnancy. Pregnant mice lacking the IFN-α/β receptor (IFNAR) became viremic and had higher mortality compared with nonpregnant animals. Notably, an embryo with functional IFN signaling alone was sufficient to rescue the pregnant IFNAR$^{-/-}$ dam from virus-associated demise. Placental IFN was also an important regulator of viral replication in placental tissue and significantly affected viral transmission to the fetus. These findings highlight the role of fetal/placental IFN in the modulation of viral infection in the mother and fetus. *The Journal of Immunology*, **2017, 198: 3029–3032.**

The maternal immune system is significantly affected by pregnancy. The changes in immune function can result in the beneficial amelioration of some autoimmune disorders but can also impact the severity of the responses to some infections (1). For example, pregnant women are more susceptible to infection with *Toxoplasma gondii* (2–4), *Plasmodium falciparum* (5), and *Listeria monocytogenes* (6). They also exhibited increased susceptibility and/or disease severity associated with numerous types of viral infections. For example, pregnant women have higher mortality associated with varicella virus infection, which is 10 times more likely to be complicated by pneumonia during pregnancy (1, 7, 8). They are more susceptible to rubeola (measles), and the infection is more likely to cause death (9, 10). Furthermore, during the 2009 H1N1 influenza pandemic, pregnant women developed more severe flu-related complications, such as hospitalization and death, compared with the general population (11–19). These same symptoms were also confirmed to have occurred during the 1918 H1N1 (20, 21) and 1957 H5N1 (22, 23) pandemics. Despite these clear differences, there is still little known about pregnancy-associated changes in the immune response to pathogens.

Several viruses were shown to infect the placenta, including CMV, HSVs, human papillomaviruses, Lassa fever, and Zika virus (24–26). The placenta itself is a robust immune organ that expresses pattern recognition receptors and consequently responds to these pathogens at the maternal–fetal interface (27–33). Activation of these pattern recognition receptors by viral proteins can result in activation of the transcription factor NF-κB and/or type I IFN. Type I IFN, through the IFN-α/β receptor (IFNAR), induces a multitude of IFN-stimulated genes that can block viral replication and activate the immune system in response to the virus (34, 35). Interestingly, many viruses can target the IFN pathway, and successful inhibition enhances infection and viral spread. Specifically, we showed that, in our animal model of pregnancy and herpesvirus infection, murine herpesvirus-68 (MHV68) infects the placenta (28, 36–38) and inhibits the expression of type I IFN (28). The inhibition of IFN results in an enhanced proinflammatory response to bacteria (37, 38), demonstrating that placental IFN is an important mediator of the immune response. Could these changes in placental immune modulation also affect maternal well-being?

We hypothesize that fetal–placental IFN signaling is critical for the protection of the fetus against viral infection and can also significantly affect maternal health. This could be due to changes in placental modulation of the maternal immune response, or the presence of the placenta could be an additional source of an infectious virus, thus enhancing disease

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Received for publication October 31, 2016. Accepted for publication February 13, 2017.

This work was supported by National Institutes of Health Grant 3N01 HD23342 and by the Intramural Research Program of the Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Department of Health and Human Services.

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Abbreviations used in this article: D, protocol day; E, embryonic day; IFNAR, IFN-α/β receptor; MHV68, murine herpesvirus-68; wt, wild-type.

www.jimmunol.org/cgi/doi/10.4049/jimmunol.1601824

severity. Specifically, we suggest that viral inhibition of placental IFN is a key mediator of these changes. To test our hypothesis, in this study we compared disease severity between pregnant and nonpregnant mice without the type I IFNR to determine whether IFN contributes to pregnancy-specific mortality during viral infections. Furthermore, we investigated viral replication and the severity of the maternal and fetal response to viral infections by manipulating embryonic–placental IFN. We demonstrate that loss of fetal-derived type I IFN, specifically, contributes to fetal viremia, and fetal–placental IFN signaling can affect maternal mortality.

Materials and Methods

MHV68 production and quantification

MHV68 passage and viral titer determination has been described previously (37, 39). Results are reported as copies per 100 or 500 ng of DNA.

Animals: viral infection

All animals were maintained in the Yale University School of Medicine Animal Facility under specific pathogen–free conditions. All procedures were approved by the Yale University Institutional Animal Care and Use Committee, and between 6 and 10 mice were used in each group to obtain statistical power. IFNAR$^{-/-}$ and C57BL/6 mice were obtained from the Jackson Laboratory (Bar Harbor, ME). IFNAR$^{-/-}$ (B6.129S2-Ifnar1^{tm1Agr}/Mmjax) mice were backcrossed onto C57BL/6J for three generations preceding experiments. Adult mice (8–12 wk of age) with vaginal plugs were infected i.p. at embryonic day (E)8.5 postconception with 1×10^5 PFU MHV68 in 100 μl of DMEM or DMEM alone (vehicle). Viral infection was quantified by analyzing the copy number for MHV68 ORF53 using quantitative PCR (39).

Animals: breeding crosses and genotyping

To produce litters with IFNAR$^{+/-}$ and IFNAR$^{-/-}$ pups, IFNAR$^{-/-}$ males were bred to IFNAR$^{+/-}$ females. Animals were infected with MHV68 at E8.5, as described above, and sacrificed at E15.5 to determine viral titers in placentas and fetal samples. Each fetus was paired with its placenta and genotyped using the genotyping protocol described for stock number 32045-JAX through the Jackson Laboratory. The following primers were used: common (9850): 5′-CGAGGCGAAGTGGTTAAAAG-3′; wild-type (wt) reverse (9851): 5′-ACGGATCAACCTCATTCCAC-3′; and mutant (oIMR8963): 5′-AATTCGCCAATGACAAGACG-3′.

Animals: embryo transfers

C57BL6/J served as donor animals, and IFNAR$^{-/-}$ were pseudo-pregnant recipients. Donor mice, 3–4-wk-old weanlings, received 5 IU pregnant mare serum gonadotropin (National Hormone and Peptide Program) on protocol day (D)1, followed by 5 IU human chorionic gonadotropin (CG10; Sigma) 46 h later, on D3. Subsequently, donor females were placed with intact C57BL6/J males overnight (1:1 ratio) and were removed by 9 a.m. on D4. Pregnancy was confirmed by observation of vaginal mucus plugs (E0.5). The recipient females (10–12 wk old) were bred with vasectomized males on D5 and were removed the following morning (D6). Initiation of pseudo-pregnancy in recipients was confirmed by the evidence of a vaginal mucus plug on the morning of D6, pseudo-pregnant day 0.5. E3.5 blastocysts with intact zonas were flushed from the uteri of E3.5 donors with M2 medium (Ambion). Blastocysts were washed by moving into three separate microdrops of M2, and placed in a microdrop of KSOM (Ambion) under embryo-tested oil (Irvine Scientific). Between 16 and 20 embryos were transferred into P2.5 recipients using a transcervical, nonsurgical embryo transfer device (ParaTechs).

Statistics

Differences between means (three groups or more) were determined using ANOVA, and differences between two groups were analyzed using independent *t* test functions of GraphPad inStat statistical software (GraphPad, La Jolla, CA). Differences under curves were analyzed with GraphPad to determine statistical differences in survival. A p value ≤ 0.05 was considered significant, and data are presented as mean ± SEM.

Results and Discussion

Mortality is higher in pregnant ifnar$^{-/-}$ *mice compared with nonpregnant* ifnar$^{-/-}$ *mice infected with MHV68*

MHV68 is a natural murine pathogen that is not lethal to wt mice (38). However, nonpregnant *ifnar*$^{-/-}$ mice are more susceptible to MHV68 infection and show ~50% mortality within 6 wk of infection. The susceptibility of pregnant wt or *ifnar*$^{-/-}$ mice to MHV68 is unknown. To address this issue, we infected pregnant (E8.5) and nonpregnant C57BL6/J (wt) and *ifnar*$^{-/-}$ mice with 10^5 PFU MHV68 and monitored their responses. Infected pregnant wt mice did not show any sign of illness, and all survived (Fig. 1A). Strikingly, 100% of the pregnant *ifnar*$^{-/-}$ mice succumbed within 5 d postinfection with MHV68 (Fig. 1B). Similar to previous reports, the nonpregnant *ifnar*$^{-/-}$ mice did not show any sign of mortality during the same period (Fig. 1B). We observed a decrease in survival only after 10 d postinfection (Fig. 1B)

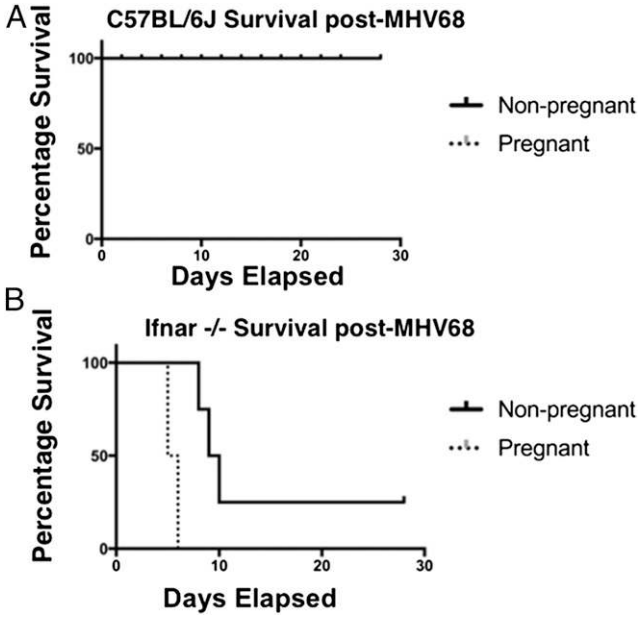

FIGURE 1. Loss of IFNAR results in higher mortality in pregnant mice compared with nonpregnant mice infected with virus. All pregnant animals were infected with 10^5 PFU MHV68 i.p. on E8.5. (**A**) C57BL6/J pregnant versus nonpregnant survival (n = 10). (**B**) *ifnar*$^{-/-}$ pregnant versus nonpregnant survival (n = 8).

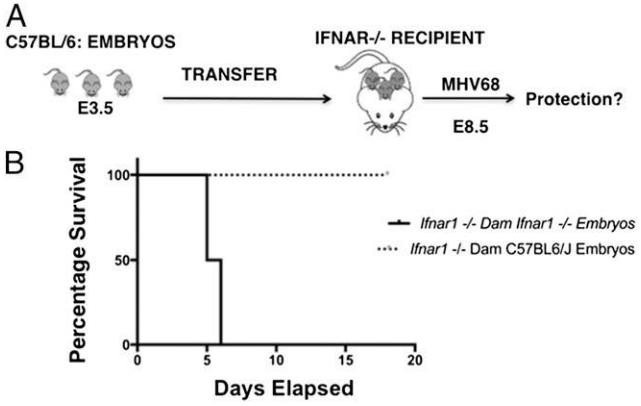

FIGURE 2. Placental IFN is sufficient to protect IFNAR$^{-/-}$ mothers from virus-associated mortality. (**A**) Experimental design: wt embryos were transferred into pseudo-pregnant *ifnar*$^{-/-}$ recipients who received MHV68 on E8.5. (**B**) wt embryos protected IFNAR$^{-/-}$ dams from virus-induced mortality (n = 6).

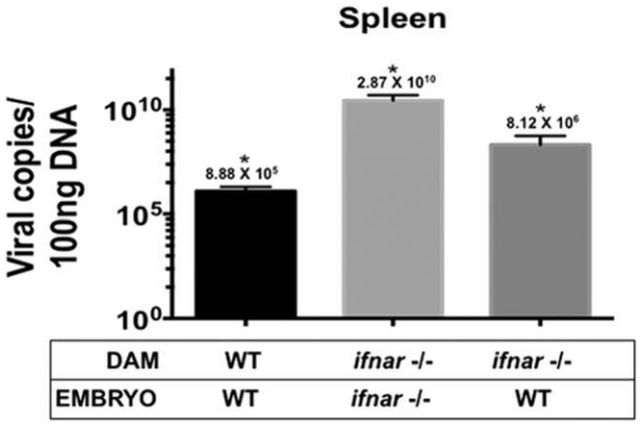

FIGURE 3. Effect of placental IFN on maternal viral titers. Maternal viral infection was evaluated in the spleen of wt and *ifnar*$^{-/-}$ dams carrying wt or *ifnar*$^{-/-}$ embryos. The dam's splenic MHV68 titers are 8.88×10^5 when the embryo and dam are wt, the dam's splenic MHV68 titers are 2.87×10^{10} when the embryo and dam are *ifnar*$^{-/-}$, and the dam's splenic titers are reduced to 8.12×10^6 when the embryo is wt and the dam is *ifnar*$^{-/-}$ ($n = 5$). *$p = 0.0314$.

(40, 41), demonstrating that the loss of type I IFN signaling during pregnancy was more detrimental to pregnant animals.

Placental and fetal IFN signaling can protect the mother from fetal viremia in the absence of maternal IFNAR

Because one obvious difference between pregnant and nonpregnant mice is the presence of the placental/fetal unit, we next determined whether the loss of IFN signaling in the embryo/placenta, specifically, contributed to the increased mortality observed in the pregnant *ifnar*$^{-/-}$ mice. For this, wt embryos were transferred into pseudo-pregnant *ifnar*$^{-/-}$ mice who were infected with MHV68 at day E8.5, and animals were monitored for signs of illness or mortality (Fig. 2A). Strikingly, the presence of wt embryos completely prevented MHV68-induced maternal mortality of pregnant *ifnar*$^{-/-}$ mice (Fig. 2B). Further, the *ifnar*$^{-/-}$ mothers with wt embryos had significantly lower viral titers in the spleen (10,000 times lower) compared with *ifnar*$^{-/-}$ mothers carrying *ifnar*$^{-/-}$ pups. The viral titers observed in the *ifnar*$^{-/-}$ mothers with wt embryos were similar to splenic titers observed in wt mothers and embryos (Fig. 3).

Loss of fetal–placental IFNAR results in viral transmission to the fetus

To test the role of fetal–placental IFNAR signaling in controlling local viral replication, we infected wt and *ifnar*$^{-/-}$ mice at E8.5 and compared viral titers of the placentas and fetuses at E15.5. As expected, wt animals had significantly lower placental titers compared with *ifnar*$^{-/-}$ mice (Fig. 4A); furthermore, there was no viral transmission to wt fetuses (Fig. 4B). In contrast, we found significant MHV68 viral titers in the placenta and fetus of *ifnar*$^{-/-}$ mice (the viral titers exceeded 10^{10} in *ifnar*$^{-/-}$ placentas and fetuses) (Fig. 4).

Next, we bred *ifnar*$^{-/-}$ males with *ifnar*$^{+/-}$ females. This resulted in litters with half of the embryos/placentas lacking a functional IFNAR (homozygous *ifnar*$^{-/-}$) in a mother with functional IFNAR (*ifnar*$^{+/-}$) and half of the embryos/placentas with a functional IFNAR (heterozygous *ifnar*$^{+/-}$) in a heterozygous (*ifnar*$^{+/-}$) mother. Pregnant females were infected with MHV68 on E8.5; at E15.5 the fetal–placental

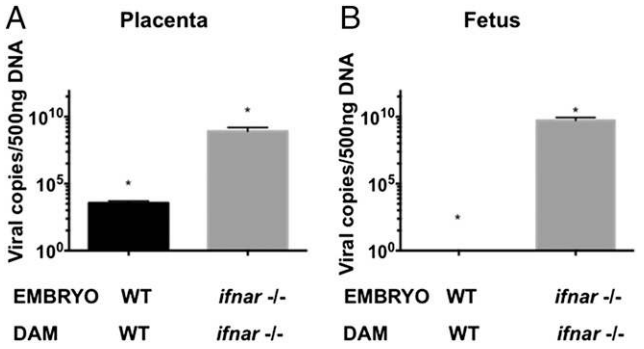

FIGURE 4. Type I IFN inhibits placental viral infection and viral transmission to the fetus. wt and *ifnar*$^{-/-}$ pregnant mice were exposed to MHV68 on E8.5, and viral titers were determined on E15.5. Placental (**A**) and fetal (**B**) MHV68 titers are significantly higher in *ifnar*$^{-/-}$ mice compared with wt mice ($n = 6$). *$p < 0.001$.

units were collected and genotyped, and viral titers were quantified in the individual placentas and fetuses. Although the mothers had a functional IFNAR, viral titers were significantly higher in placenta and fetus lacking IFNAR signaling (homozygous *ifnar*$^{-/-}$) compared with those with a functional IFNAR (heterozygous *ifnar*$^{+/-}$) (Fig. 5). Therefore, despite sharing the same maternal phenotype (functional IFNAR), the susceptibility to infection was increased in fetal/placenta units lacking a functional IFNAR (Fig. 5).

In summary, this study demonstrates that placental/fetal IFN signaling regulates fetal viral transmission and has the potential to modulate maternal viral infection. It is well established that many viruses inhibit IFN signaling as a mechanism to evade the host immune response. Therefore, a virus that successfully inhibits placental IFN could be much more effective at infecting the fetus. We also provided evidence that the fetus/placenta can affect the mother by demonstrating that fetal/placental IFN signaling was sufficient to protect the mother from viremia and death. We postulate that if placental IFN is inhibited, this could also affect placental function and placental regulation of the maternal immune response; this is the basis of ongoing studies.

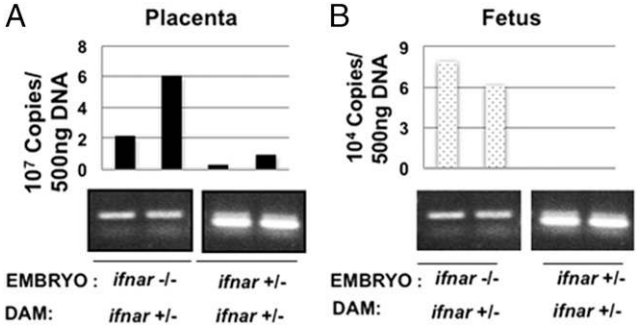

FIGURE 5. Placental type I IFN regulates viral transmission to the placenta and fetus. *ifnar*$^{+/-}$ females were bred with *ifnar*$^{-/-}$ males. Pregnant mice were infected with MHV68 at E8.5, and embryos were collected and genotyped at E15. Representative samples of embryonic genotypes from IFNAR$^{-/-}$ embryos (single band) or IFNAR$^{+/-}$ embryos (double band) and associated viral titers in their respective placentas (**A**) and fetuses (**B**). Viral titers from homozygous *ifnar*$^{-/-}$ and heterozygous *ifnar*$^{+/-}$ embryos. IFNAR$^{-/-}$ embryos have higher viral titers, even when the mother is a heterozygote ($n = 5$–7).

Acknowledgments

We thank A.F. Parlow, Scientific Director, Los Angeles Biomedical Research Institute National Hormone and Peptide Program, for the pregnant mare serum gonadotropin used for superovulation protocols for embryo-transfer experiments. Also, we extend our gratitude to JoAnn Bilyard for the careful editing of this manuscript.

Disclosures

The authors have no financial conflicts of interests.

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