IFN-g Deficiency Hinders Resolution of CNS Pathology Caused by Zika Virus Infection.

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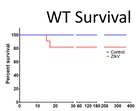
Abstract

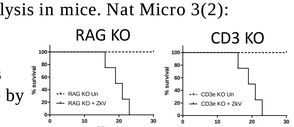
Perinatal exposure to Zika virus (ZkV) is associated with developmental abnormalities and life-long neurological defects that vary in magnitude depending on the time of infection. Several studies show that NK cells and CD8+ T cells are critical for viral clearance, however these cytolytic cells may also contribute to the pathology. Interferon gamma (IFNg) is central to both NK and CD8 T-cell function, and previous viral meningoencephalitis studies suggest that it contributes to neurodegeneration by maintaining activated microglia and stimulating CXCL10 secretion. Our lab has developed a model of ZkV pathogenesis in neonatal C57Bl mice, where viral challenge leads to apoptosis and extensive neurodegeneration that are associated with severe but transient neurological symptoms including ataxia, seizures, and paralysis. In this model, NK and CD8 T-cells are critical for survival and mice that lack either cell population fail to limit the infection and do not survive. To explore the role of IFNg in viral control and/or pathogenicity, we infected neonatal IFNg KO mice with ZkV. We show that IFNg deficient mice have an impaired survival rate (55-65 %), increased viral load with a broader distribution of virus throughout the brain, and more extensive apoptosis leading to cortical thinning which is not evident in the WT mice. Although there are no differences in total infiltrating cells, T-cells, or macrophage populations between the genotypes at the peak of inflammation, 15 dpi, the NK cell population in the IFNg KO mice is halved. This inadequate NK cell response in the IFNg KO's is associated with lower gene expression levels of granzyme b and reduced MHC class I and II expression. By 30 dpi ZkV is reduced to similar levels in both genotypes, but higher levels of chemokines CCL5, CXCL10, and CD3e (T cell marker) remain in the IFNg KO mice. The broader viral distribution in the CNS of IFNg KO mice during the acute phase of disease and the asynchronous immune response suggests that IFNg is indispensable in containing viral dispersion and inflammation. Lastly, the more extensive parenchymal damage in IFNg KO mice was associated with more severe behavioral and motor defects compared to the WT counterparts. This work examines the role of IFN-g on neurological damage and long-term sequelae in mice infected with Zika virus. Improved understanding of Zika neuropathology can inform therapies to reduce the impact of the disease.

Background

- Our lab has shown that Zika (PRV59) virus (ZkV) infection on postnatal day 1 (P1) in B6WT mice causes neurological sequelae that largely subside by P30 mimicking Congenital Zika Syndrome.



- IFNγ is a powerful cytokine important to resolving many viral infections including Measles virus and Herpes Simplex 1 virus, and is produced by T-cells and NK cells
- CD8+ T-cells are important for Zika virus clearance and may be responsible for symptomatology. Iwasaki et al. (2018). Antiviral CD8 T Cells induce Zika-virus-associated paralysis in mice. Nat Micro 3(2): 141-147
- RAG KO mice have no T-cells or B-cells and 100% succumb to P1 ZkV challenge by P25.



Acknowledgements

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- ORISE

Results 1: Survival rates, viral load, and symptomatology of P1 infected B6WT and IFNγ KO mice

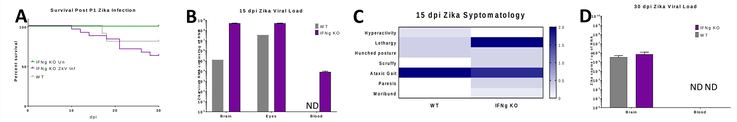

Figure 1. IFNγ KO mice have impaired survival upon P1 challenge with ZkV. (A) Less than 65% of IFNγ KO mice survive P1 challenge. **(B)** They have a higher viral load compared to B6WT in immune privileged sites and detectable viral RNA in peripheral blood. **(C)** At 15 dpi, gamma deficient mice are more lethargic and have higher incidence of paresis and becoming moribund. (D) By 30 dpi there is still twice as much viral RNA in IFNγ KOs, but no virus is detectable in the periphery.

Results 2: 15dpi Zika virus, infiltrating immune cells, and apoptosis distribution

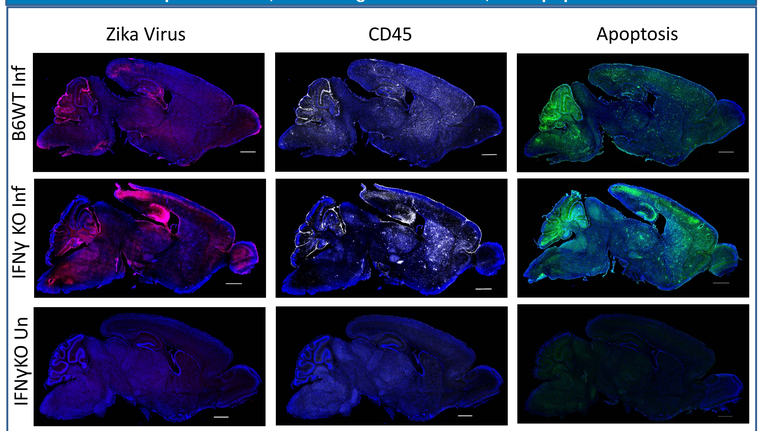

Figure 2. IFNγ KO show increased viral distribution and apoptosis.

Results 3: 30 dpi Zika virus, infiltrating immune cells, and apoptosis distribution

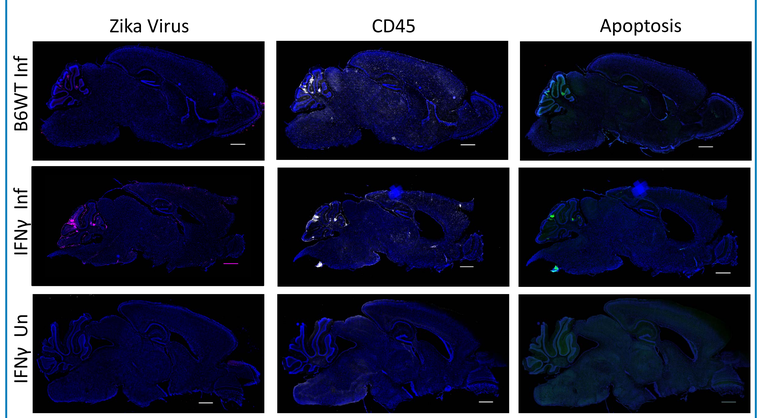

Figure 3. Viral load and apoptosis remain higher in surviving IFNγ KO mice than in WT mice.

Results 4: Infiltrating cell diversity and activation at 15 dpi

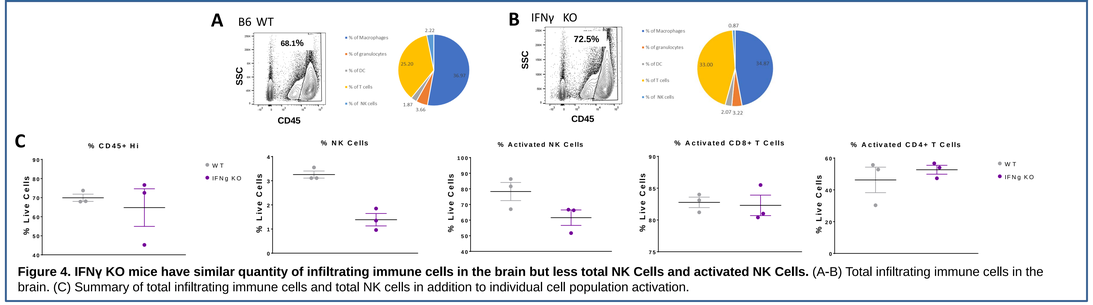

Figure 4. IFNγ KO mice have similar quantity of infiltrating immune cells in the brain but less total NK Cells and activated NK Cells. (A-B) Total infiltrating immune cells in the brain. (C) Summary of total infiltrating immune cells and total NK cells in addition to individual cell population activation.

Results 5: Gene Expression at 15 dpi

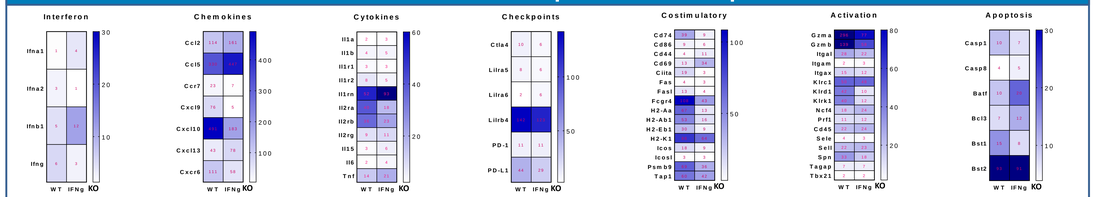

Figure 5. At 15dpi the CNS, IFNγ KOs show similar expression of some inflammation markers but reduced NK cell activation markers compared to WT mice.

Results 6: Gene Expression at 30 dpi

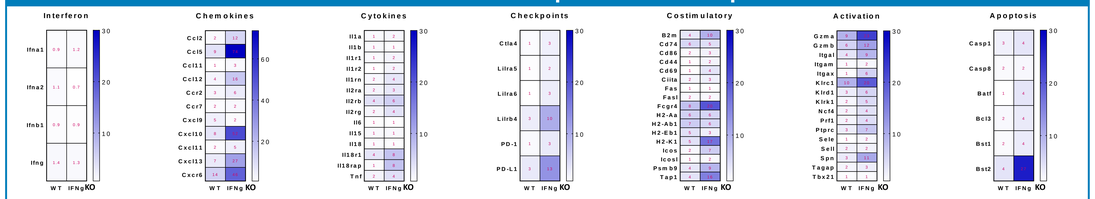

Figure 6. At 30 dpi, surviving IFNγ KO mice show increased expression of markers for infiltrating cells and inflammation than infected WT mice.

Result 7: 180 dpi infiltrating cells and behavioral differences

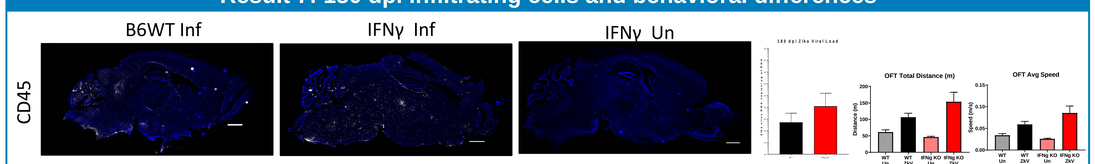

Figure 7. Surviving IFNγ KO mice have a greater distribution and presence of immune infiltrating cells by immunofluorescence staining, higher viral loads by qPCR, and increased hyperactivity in behavioral studies

Conclusion

- IFN$_\gamma$ is needed to mount an effective response to ZkV.
- IFN$_\gamma$ KO mice have reduced NK cell activation markers that correlate with reduced viral clearance and increased loss of brain mass.
- Long term, the absence of IFN$_\gamma$ is associated with increased viral RNA, increased CD45$^+$ cell distribution in the brain and worse motor and behavioral function