

Effects of type 1 interferon deficiency on B-cells in lupus-prone mice



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Introduction

- Systemic Lupus Erythematosus (SLE) is a debilitating autoimmune disease that affects over 5 million people worldwide
- Lupus occurs more frequently in females than in males (8:1)
- Disease determining factors include genetics, environmental factors and sex hormones
- Symptoms of SLE in patients vary and can have multiple affects including anemia, hair loss and glomerulonephritis
- Deficiency in IFNAR ameliorates disease (Jorgensen et al, 2007) and elevating endogenous type I interferons, accelerates disease development (Jorgensen et al, 2006).

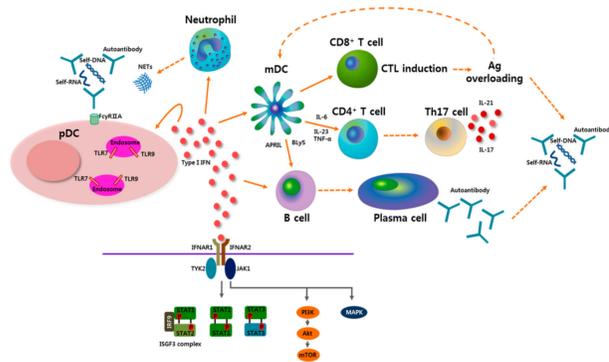


Figure 1: Plasmacytoid dendritic cell and associated lupus pathways (1)

- Type 1 interferon is produced from plasmacytoid dendritic cells through TLR7 and TLR9 activation and signals through IFNAR
- IFNAR activation in B cells leads to autoantibody production in SLE patients
- B6.Nba2 backcrossed mice develop a lupus-like disease

Hypothesis

Specific deletion of IFNAR in B cells will decrease autoantibody production, immune complex formation and deposition

Materials and Methods

- Cre recombinase is a reliable method used in IFNAR gene knockout that recognizes and splices specific DNA sequences (LoxP sites)

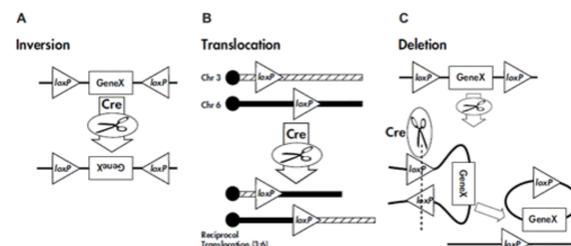


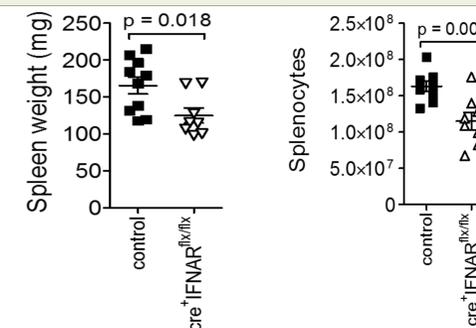
Figure 2: Cre recombinase methods of use (2)

- Genotyping by ear clipping: Digestion, DNA extraction, PCR, Gel-electrophoresis
- Flow Cytometry was used for the identification of cell subsets/subpopulations
- Enzyme-linked immunosorbent assay (ELISA) was used to measure total IgG, IgM, and anti-chromatin IgG concentrations.
- Immunofluorescence staining of kidney samples with IgG-TxRd (1:500) and C3-FITC (1:500)
- Project sample size of B6.Nba2 mice:

IFNAR +/+ : n=6
IFNAR flx/+ : n=3
IFNAR flx/flx : n=9

Results

IFNAR flx/flx mice exhibit lower spleen weight and splenocyte count than IFNAR +/+ and flx/+ mice



IFNAR flx/flx mice show similar deposition of IgG than IFNAR +/+ and flx/+ mice

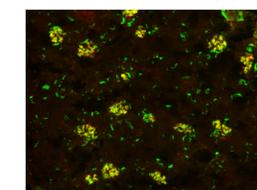
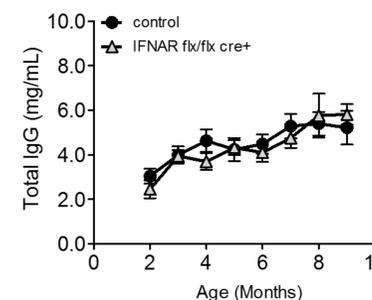


Figure 3: IFNAR flx/flx kidney samples taken at 9 months

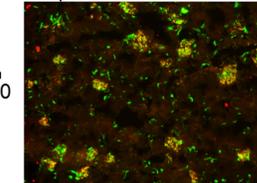


Figure 4: IFNAR +/+ kidney samples taken at 9 months

IFNAR flx/flx mice show similar deposition of IgM/C3 than IFNAR +/+ and flx/+ mice

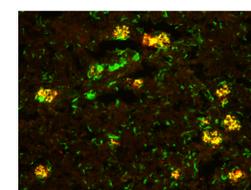


Figure 5: IFNAR flx/flx kidney samples taken at 9 months

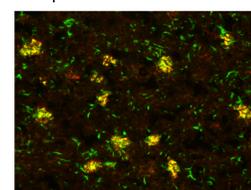
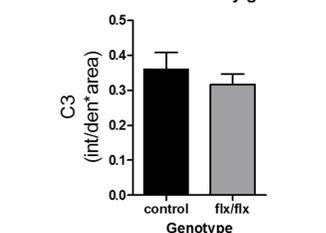
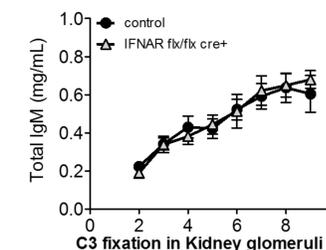
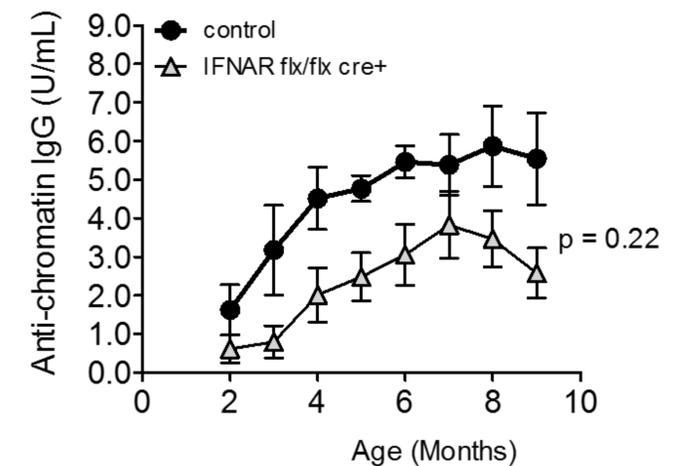


Figure 6: IFNAR +/+ kidney samples taken at 9 months



IFNAR flx/flx mice exhibit a lower concentration of serum anti-chromatin IgG antibodies than control mice



Conclusions

B-cell specific IFNAR deficiency in B6.Nba2 lupus-prone mice results in:

- Significantly lower spleen weight
- Significantly lower splenocyte count
- Significantly lower anti-chromatin IgG levels
- But, no difference in immune complex deposition and C3 fixation.

Acknowledgments

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References

1. Obermoser, G, and V Pascual. "The Interferon-A Signature of Systemic Lupus Erythematosus." *Lupus* 19.9 (2010): 1012-1019. *PMC*. Web. 24 July 2016.
2. "Cre Recombinase." Reagents For the Life Sciences Industry. N.p., n.d. Web. 23 July 2016.