ANTIGEN DENSITY CAN TIP THE BALANCE BETWEEN IMMUNE SUPPRESSION AND ENHANCEMENT IN A MOUSE MODEL RELATED TO HEMOLYTIC DISEASE OF THE FETUS AND NEWBORN

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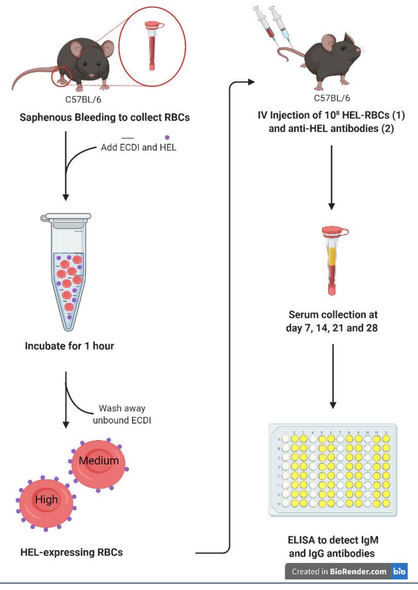
Introduction

- Maternal red blood cell (RBC) alloantibodies can cause hemolytic disease of the fetus and newborn (HDFN), a severe and possibly fatal neonatal disorder[1]
- HDFN caused by the RhD antigen can be effectively prevented through the administration of donor-derived anti-D[2]
- Prevention is due to a mechanism known as antibody-mediated immune suppression (AMIS)[3]
- Prophylaxis failures still occur in the clinic and there is no prevention for other clinically relevant antigens[4]
- Maternal RBC alloimmunization continues to be a leading cause of fetal anemia
- Better understanding of the factors underlying RBC alloimmunization is required to address these issues
- One factor is antigen density which has been shown to influence immunogenicity[5]

Aims

- Design a mouse model that allows us to alter the antigen density of our model antigen
- Investigate the relationship between antigen density and antibody dose used to induce AMIS

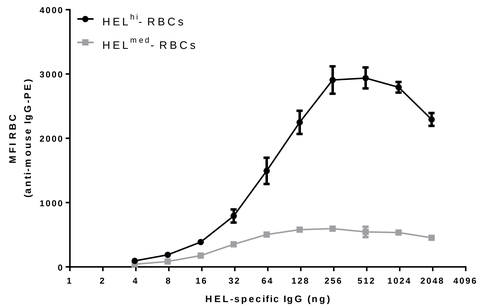
Materials and Methods



Results

1. HEL-RBCs show clear differences in antigen density

(A)

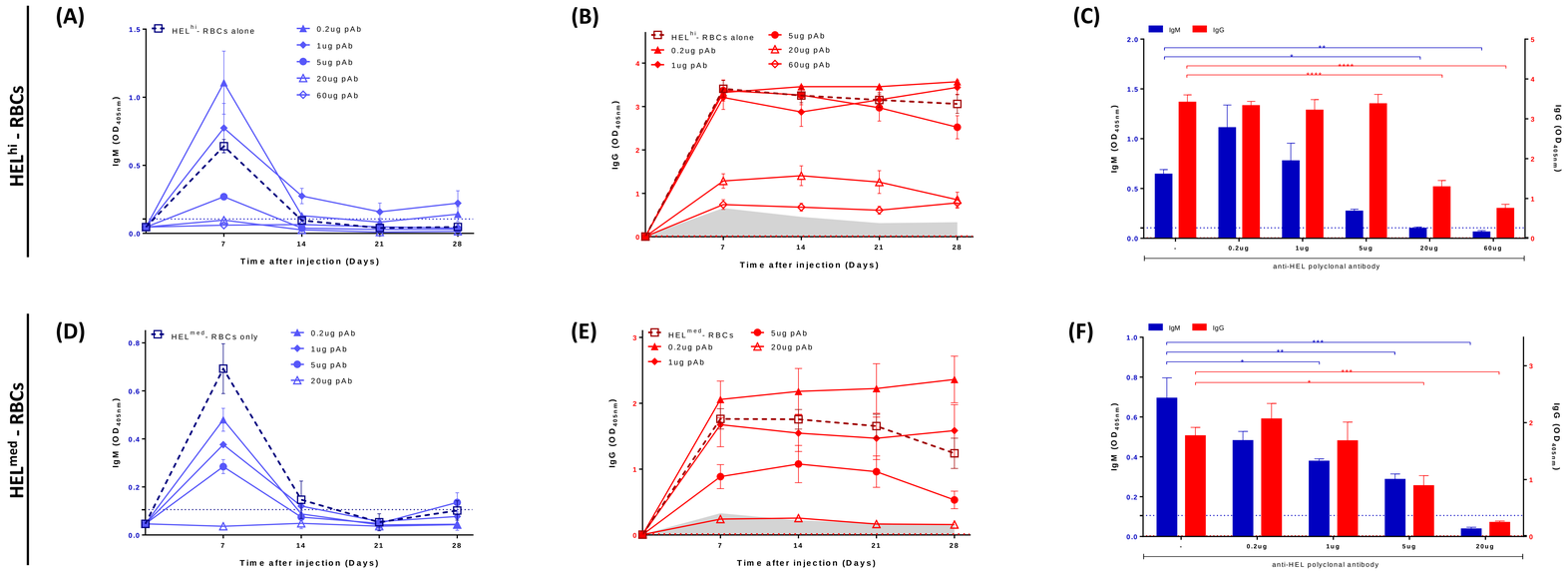


(B)

	HEL Antigen Level
HEL^{med} - RBCs	3657 ± 163
HEL^{hi} - RBCs	12402 ± 377

(A) HEL^{med} and HEL^{hi} - RBCs were incubated with serial dilutions of the monoclonal HEL-specific antibody 4B7 as indicated. The binding of 4B7 to the cells was determined by flow cytometry using a PE-labelled goat anti-mouse IgG. Data represents the mean MFI ± SEM of two independent experiments performed in duplicate. **(B)** Quantification of the HEL antigen level as assessed through the antibody binding capacity (ABC) of HEL^{med} and HEL^{hi} - RBCs. Values were obtained through the comparison of the cell's ABC to a standard curve of beads with defined and precise ABC values. Data represents the mean ± SEM of three independent experiments performed in duplicate.

2. Antigen density and antibody dose can tip the balance between AMIS and enhancement



Mice were injected with either 10^8 HEL^{hi} **(A-C)** or HEL^{med} - RBCs **(D-F)** and various concentrations of an anti-HEL polyclonal antibody (pAb). Control mice were injected with 10^8 wildtype C57BL/6 RBCs. IgM and IgG antibodies specific for HEL were analyzed via ELISA. Data represents the mean ± SEM from five different experiments with a n=5-7 for each group. Blue dotted line represents the mean ± 2 SD of the control IgM response. Red dotted line represents the mean ± 2 SD of the control IgG response. The grey area in panel B and E represent control mice that only received the highest concentration of anti-HEL pAb to determine how much residual antibody can be detected at each timepoint.

Conclusion

- Results may have important clinical implications as anti-D administration is based upon the size of the fetal bleed rather than fetal antigen density
- Administering insufficient amounts of anti-D could potentially enhance the immune response rather than suppress it
- Fetal antigen density should be considered when determining the amount of anti-D that is being administered

Acknowledgements & References

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