THE EFFECTS OF ERYTHROCYTE ANTIGEN DENSITY AND DOSE ON THE DEVELOPMENT OF IgM AND IgG ANTIBODIES IN A MURINE MODEL

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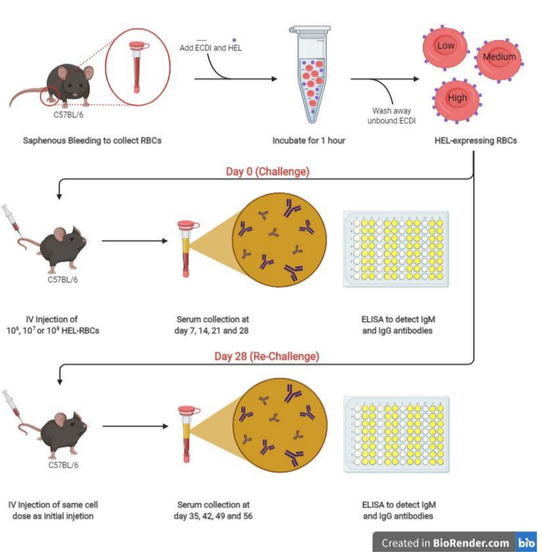
Introduction

- Exposure to foreign antigens on red blood cells (RBCs) can lead to alloimmunization and the generation of alloantibodies.
- Despite significant exposure, only 2-6% of transfusion recipients become alloimmunized[1].
- Currently, we are unable to predict which patients will make alloantibodies and are therefore responders[2].
- In addition to donor and recipient characteristics, intrinsic RBC antigen factors such as antigen density have been shown to play an important role in immunogenicity[3].
- One of the best examples of an RBC antigen that is present at different densities is RhD[4].
- Exposure to RhD during pregnancy can lead to hemolytic disease of the fetus and newborn (HDFN)[5].
- Despite this the contribution of antigen density on the development of HDFN has not been studied.

Aims

- There is currently no direct mouse model to study HDFN
- Design a mouse model that allows us to alter the antigen density of our model antigen
- Investigate the relationship between antigen density and RBC alloimmunization using this novel model

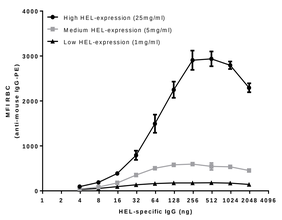
Materials and Methods



Results

1. HEL-RBCs show clear differences in antigen density

(A) (B)

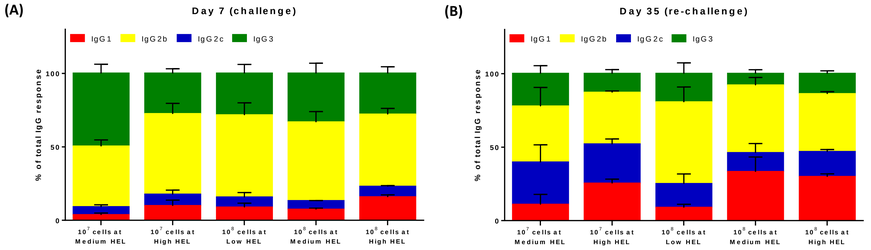


Sample	Antibody Binding Capacity
Low HEL (1mg/ml)	1240 ± 61
Medium HEL (5mg/ml)	3657 ± 163
High HEL (25mg/ml)	12402 ± 377

HEL-RBCs show consistent coupling of the different densities of HEL. **(A)** MFI of HEL-RBCs. Representative data is the mean + SEM of two independent experiments performed in duplicate. **(B)** Antibody Binding Capacity was determined using the Quantum™ Simply Cellular® Beads by Bangs Laboratory, Inc. Data represents the mean + SEM of three independent experiments.

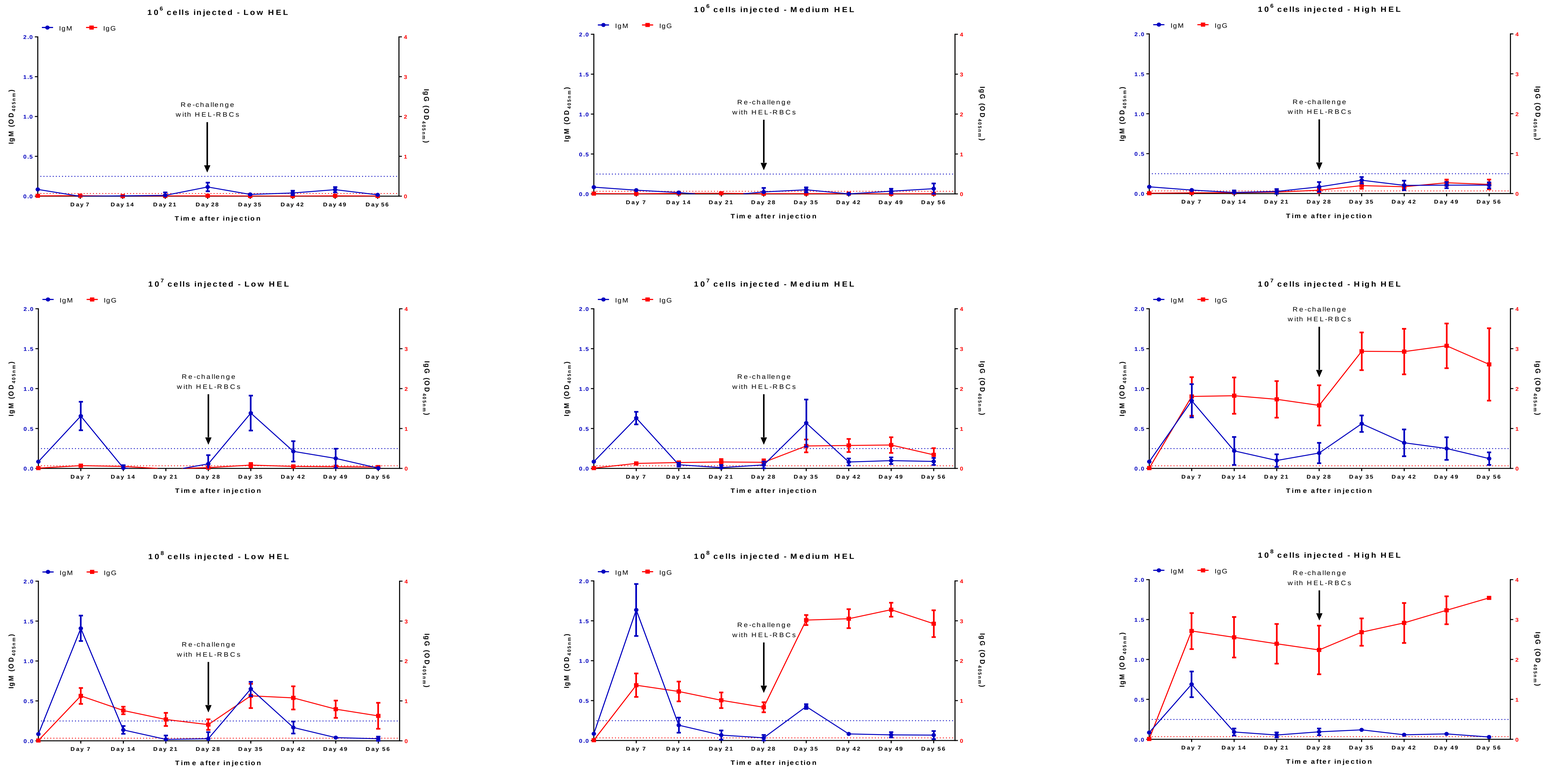
3. IgG subclass distribution shifts after repeated exposure

(A) (B)



IgG subclass distribution was analyzed via ELISA. Each subclass is represented as a percentage of the total IgG response on day 7 **(A)** and day 35 **(B)**. Data represents the mean + SEM of four independent experiments with a n=5 for each group. IgG2b and IgG3 are associated with a T-cell independent response while IgG1 and IgG2c are associated with a T-cell dependent response.

2. Productive immune responses only occur after the antigen threshold is surpassed



Mice were injected with 10^6, 10^7 or 10^8 HEL-RBCs expressing either low, medium or high densities of HEL on day 0 and day 28 (re-challenge). Control mice were injected with 10^8 wild-type C57BL/6 RBCs. IgM and IgG antibodies specific for HEL were analyzed via ELISA. Data represents the mean + SEM from four different experiments with a n=5 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.

Conclusion

- Productive immune responses only occur above an antigen threshold
- Mice can respond to low-density antigens delivered at high dosage or high-density antigens delivered at low dosage
- Weak primary immunization may not induce tolerance in mice
- IgM threshold was significantly lower than the IgG threshold
- Re-challenge increased the IgG response and altered the IgG subclass profile

Acknowledgements & References

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