The study of plasma cells and antibody responses in genetically modified mice

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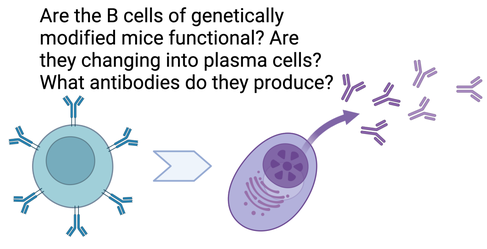
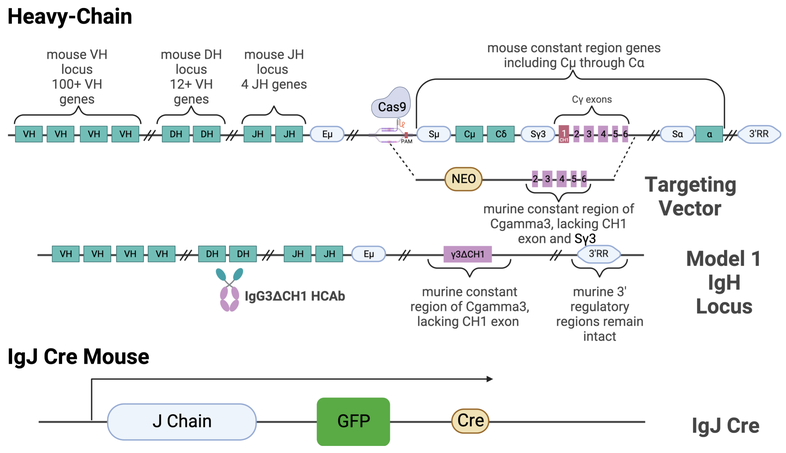
INTRODUCTION

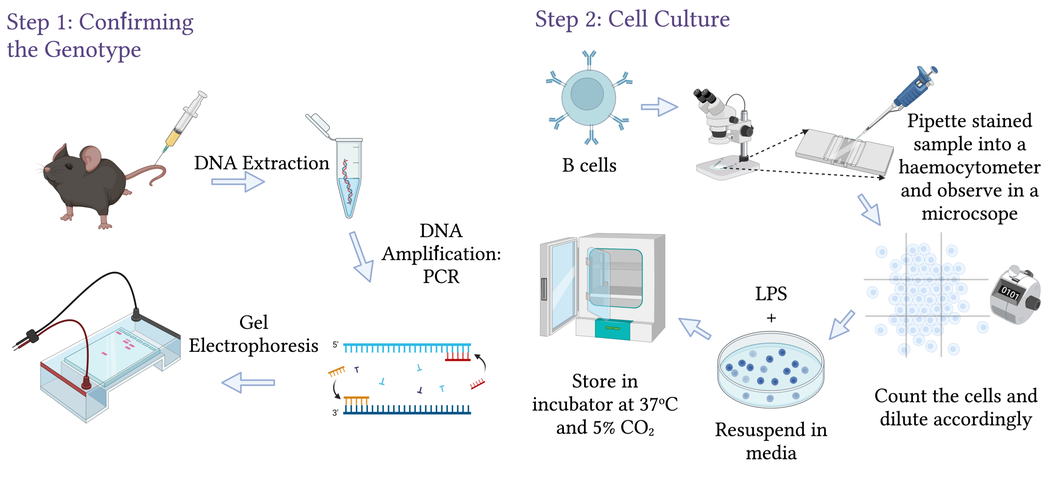
B lymphocytes produce antibodies which contribute significantly to humoral immunity. Altering mouse genetics to produce Heavy-Chain only antibodies and mechanisms of plasma cell tracking has advantages in research. The Immunoglobulin J mutation could potentially be useful trafficking method of the life of plasma cells, which is poorly understood. Our team investigated genetic mutations of heavy chain only mice as well as IgJ mouse with Cre GFP insertion near the J chain promoter (see diagram). We were interested in confirming presence of our respective mutations as well as the impact of these mutations on terminal B cell function. In order to confirm genetic mutations, we performed genotyping using PCR and gel electrophoresis. To better understand if B cell function was altered by these mutations, we put B cells into culture and stimulated them with Lipopolysaccharide (LPS) in order to promote plasma cell differentation. The heavy-chain mutated mice could potentially be useful for the production of single-chain antibodies with applications in pharmaceutical field.

Our goal was to estimate plasma cell differentiation and antibody production, by flow cytometry and ELISA, across our mutated mice and compare to wild type mice to understand if the function was altered.





METHODS



Ab	Detection			
Capture		αIgE	αIgG	αIgG3
	αIgE	✓	X	X
	αIgκ/ Igλ	X	✓	✓
	αIgG	X	✓	✓

In the ELISA, the plates were coated with three different capture antibodies - αIgE, αIgκ/Igλ and αIgG. Three different detection antibodies - αIgE, αIgG and αIgG3 - conjugated with Biotin were used. Consequently, Streptavidin-HRP were added. 'Sandwich ELISA' is more specific as it involves two antibodies. The table above exhibits which capture and detection antibodies were used together.

RESULTS

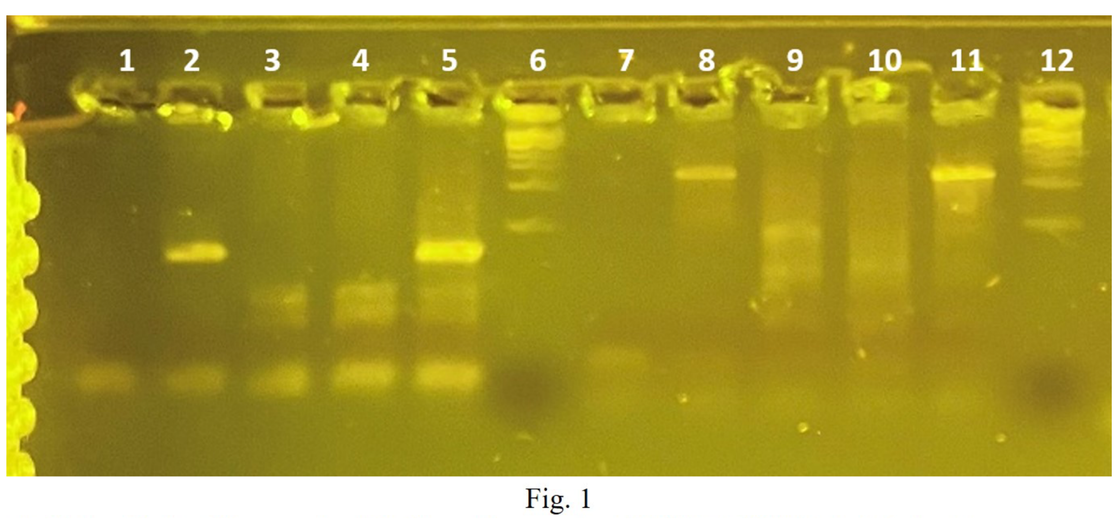


Fig. 1

1: water, 2: positive control IgJ, 3: negative control (WT), 4: WT, 5: IgJ mut, 6: ladder, 7: water, 8: positive control HC, 9: negative control (WT), 10: WT, 11: HC mut, 12: ladder

Figure 3

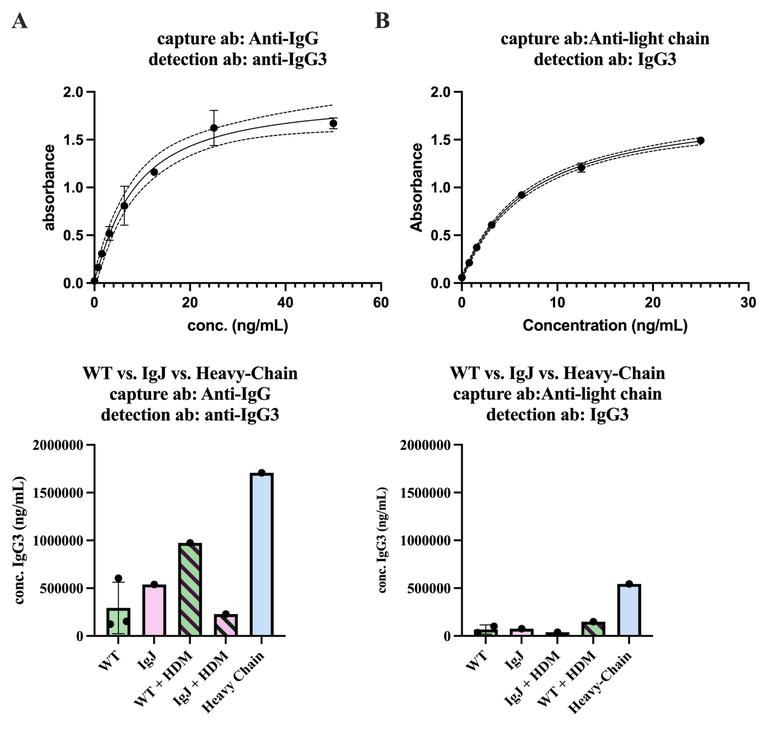
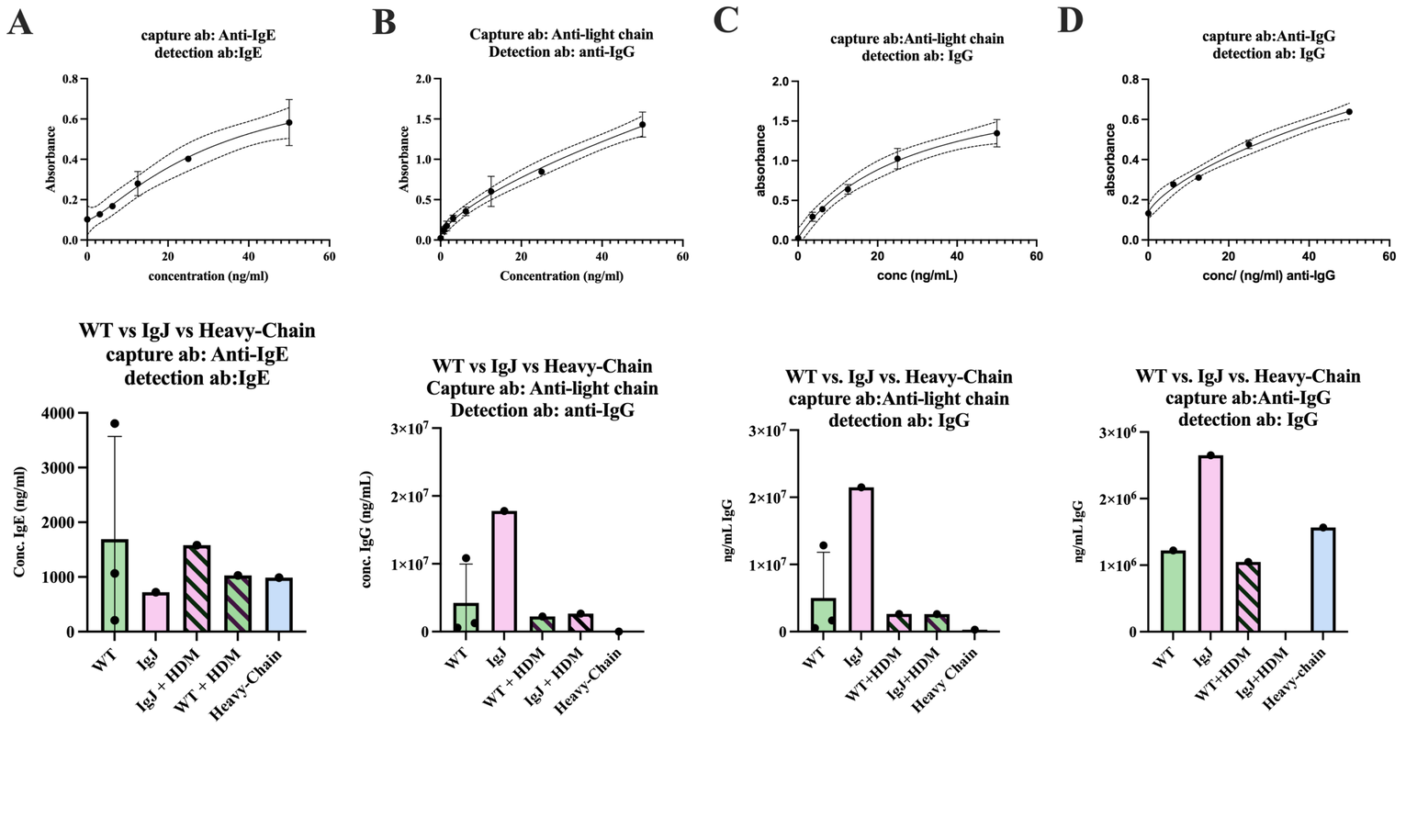


Figure 2



CONCLUSIONS

Serum IgJ versus WT				
	Detection			
Capture		IgE	IgG	IgG3
	IgE	WT>IgJ	-	-
	Igκ/Igλ	-	WT<IgJ	WT=IgJ
	IgG	-	WT<IgJ	WT=IgJ

Serum IgJ versus WT (House Dust Mite)				
	Detection			
Capture		IgE	IgG	IgG3
	IgE	WT<IgJ	-	-
	Igκ/Igλ	-	WT=IgJ	WT=IgJ
	IgG	-	WT>IgJ	WT>IgJ

Serum WT versus Heavy Chain				
	Detection			
Capture		IgE	IgG	IgG3
	IgE	WT>HC	-	-
	Igκ/Igλ	-	WT>HC	WT<HC
	IgG	-	WT<HC	WT<HC

- The IgJ mutants have higher amounts of IgG than WT. IgE levels are higher in WT compared to IgJ. IgG3 levels are equal across groups.
- HDM increases IgE in IgJ mice as expected, however not in WT.
  - HDM didn't significantly impact IgG levels.
- Heavy-chain mutation was successful. Mice have antibodies that are not captured well with light-chain, but can be captured by IgG
  - They are IgG3+
- Future work: B cell differentiation into plasma cells must be investigated further using flow cytometry
- Sample size must be increased.