T-cell dependent immunoglobulin production in miR29 knockout mice

ABSTRACT

Within the human immune system, B cells play an integral role: production of antibodies, which are essential to protect the organism from pathogens. The way to enable diversity of functions by antibodies is through class switching, a type of DNA recombination. Class switch recombination results in B cells encoding different isotypes, which are determined by distinct constant regions. One antibody isotype is IgE; these antibodies are responsible for initiation of parasite clearance, but are also implicated in allergic reactions. DNA recombination associated with class switch recombination is not random, and is heavily regulated by various factors, including T cell help and cytokine signaling. Earlier studies suggested that some of these factors are controlled by microRNAs. MiRNA regulate gene expression by binding 3' untranslated regions of target genes with complementary sequences [1]. In our investigation we focused on miR29.

In order to study the impact of miR29 we compared B cells from wild type mice to B cells with deletion of miR29, and investigated their ability to class switch and become plasma cells under a variety of conditions. These conditions are mimicking the germinal center reaction. We used IL-4 to imitate cytokine signals and CD40 for T cell help signals that B cells receive in the germinal center [2]. We aimed to discover a correlation between miR29 deletion, sensitivity to IL4, and dependance on T cell help, and the tendency of B cells to switch to IgE.

[1] Borbet, Timothy C et al. “MicroRNA regulation of B cell receptor signaling.” *Immunological reviews* vol. 304,1 (2021): 111-125. doi:10.1111/imr.13024

[2] Wade-Vallance, Adam K, and Christopher D C Allen. “Intrinsic and extrinsic regulation of IgE B cell responses.” *Current opinion in immunology* vol. 72 (2021): 221-229. doi:10.1016/j.coi.2021.06.005

HYPOTHESIS

The deletion of the miR29 in mouse B cells will cause a higher IgE production. We hypothesize that miR29 binds to the IL-4 receptor transcript and reduce its translation. We predict that by knocking out miR29 the IL-4 receptor gene is overexpressed which results in higher IgE production.

Position 5482-5488 of *Il4ra* 3' UTR	5'	...UCUGCGUGUAAUGUAUGGUGCUU...
		|||||||
miR-29a	3'	AUUGGCUAAAGUCUACCACGAU

Source: TargetScan.org

RESULTS

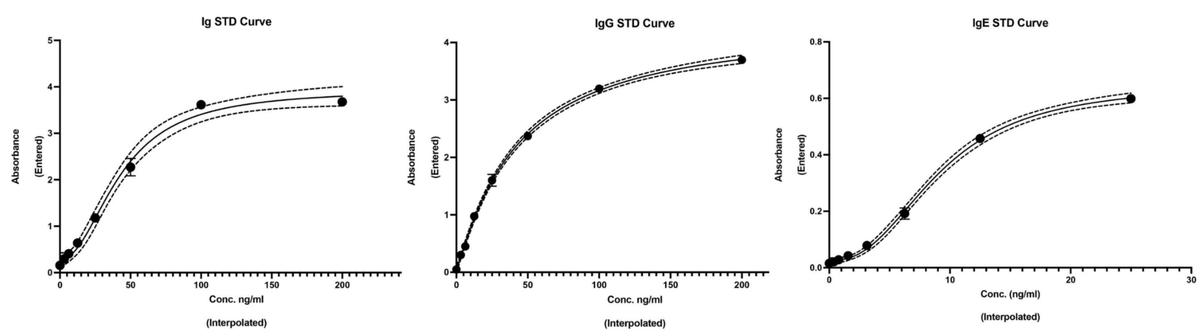

Figure 1. Standard curves for immunoglobulin quantification.

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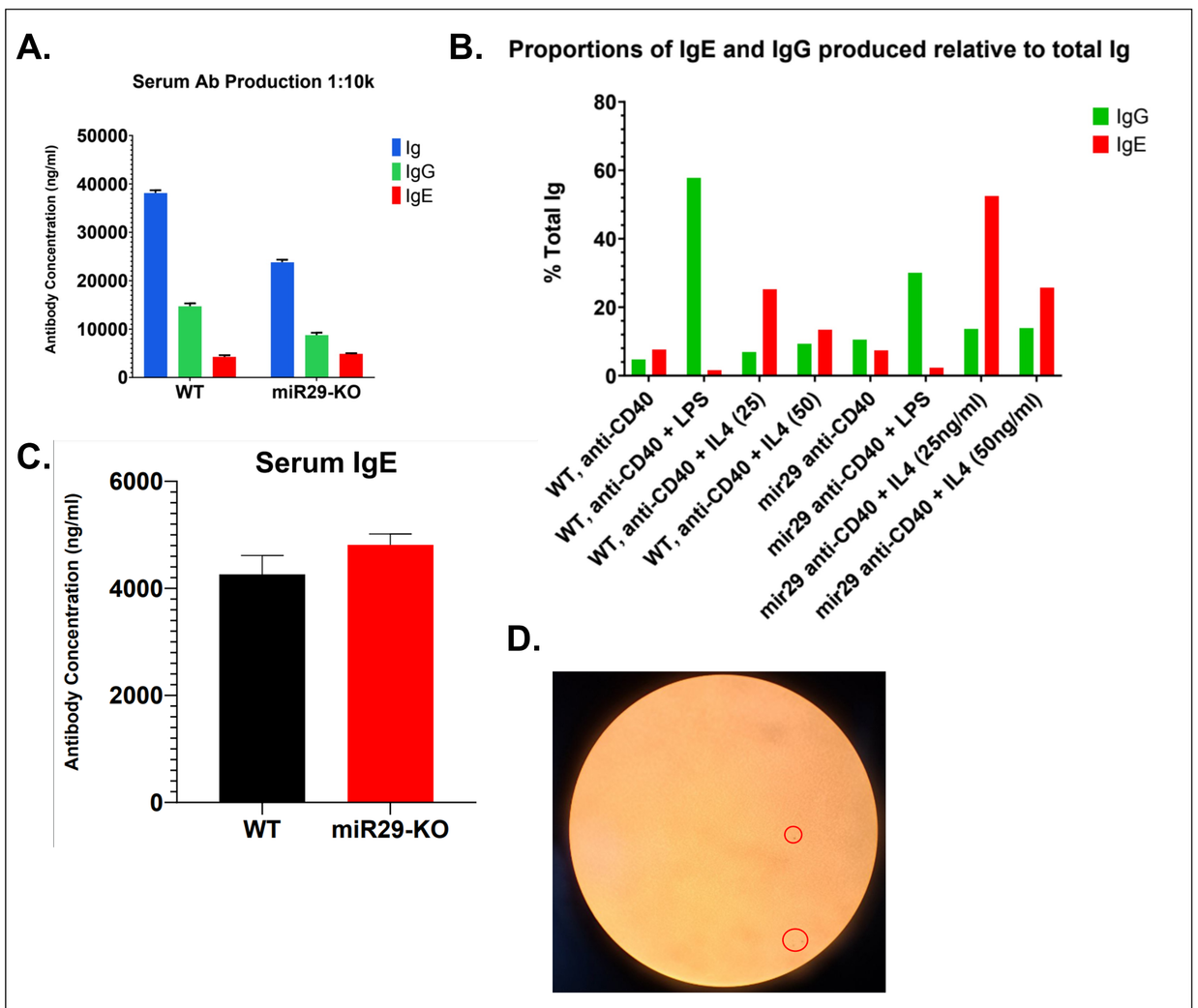
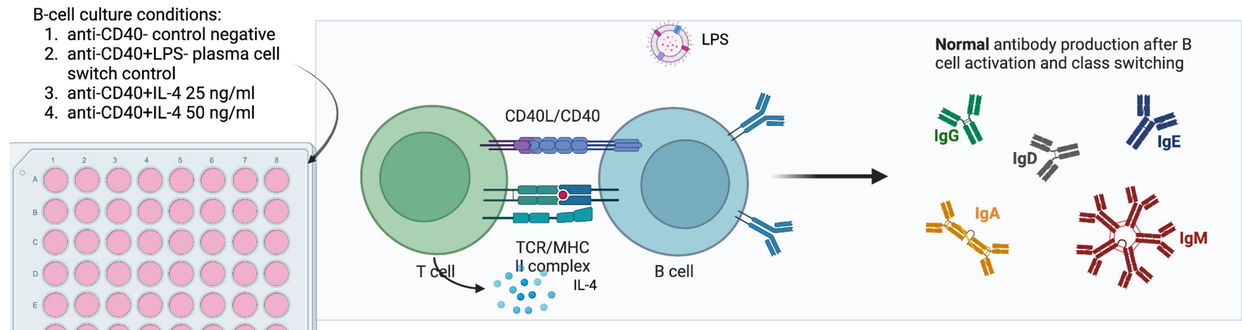


Figure 2. Standard curves for immunoglobulin quantification.

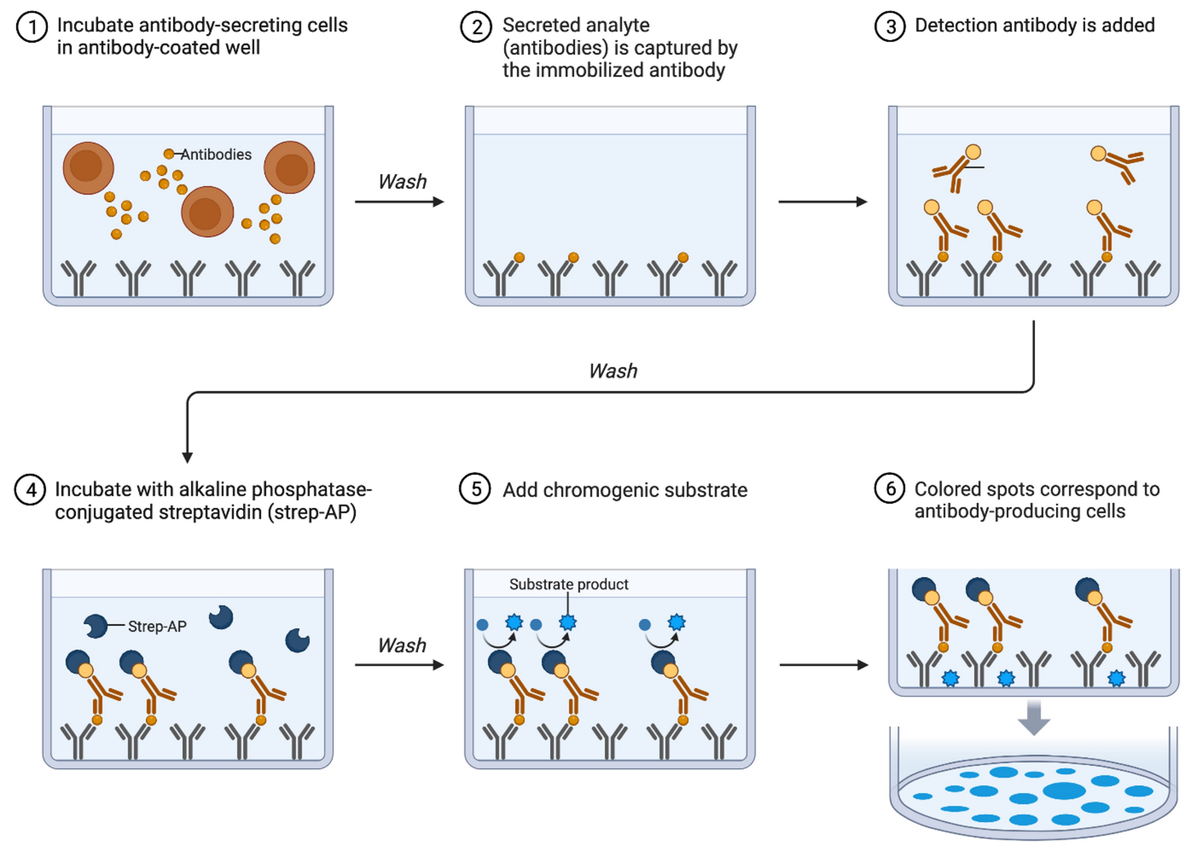
A. Serum antibody production graph
B. Proportion graph of IgE and IgG production
C. Production of IgE in serum graph
D. ELISpot plate under microscope

METHODS

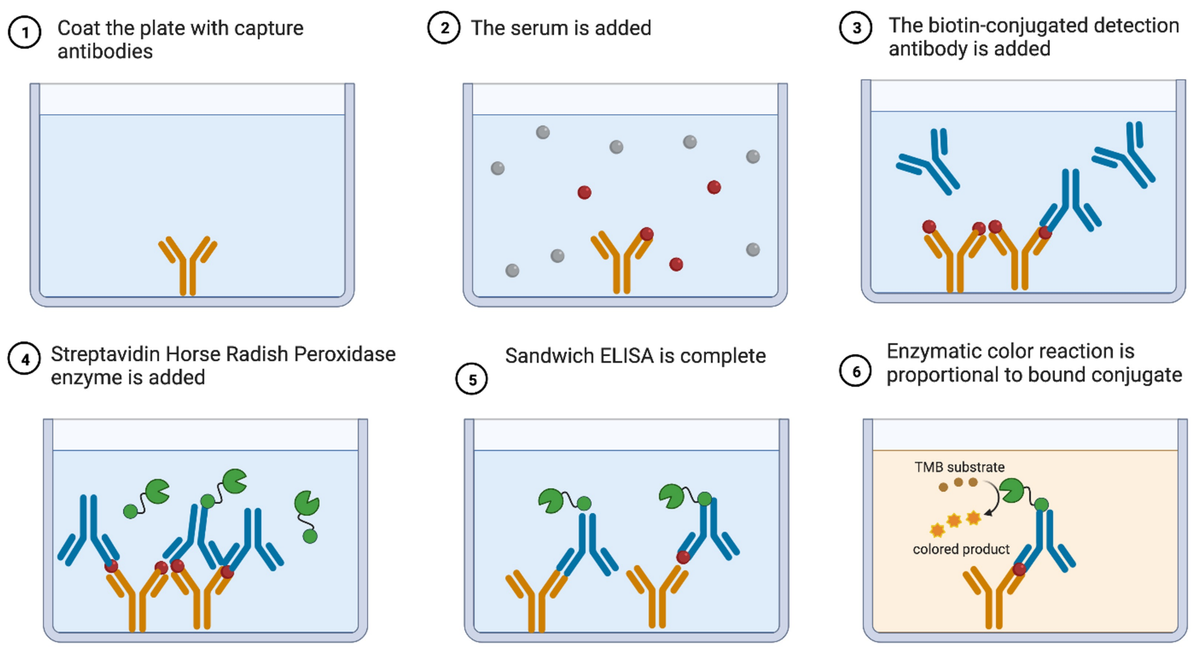
B-cell culture conditions:
1. anti-CD40- control negative
2. anti-CD40+LPS- plasma cell switch control
3. anti-CD40+IL-4 25 ng/ml
4. anti-CD40+IL-4 50 ng/ml



ELISpot - The enzyme-linked immunosorbent spot assay



ELISA - Enzyme-linked immunosorbent assay



CONCLUSIONS

After completing the ELISA and obtaining the data, our hypothesis has been supported. The deletion of the miR29 results into higher proportion of IgE across various conditions. Furthermore, IgE production is noticeably enhanced by IL-4.

Figure B shows that under anti-CD40+IL4 conditions, the miR29-KO B cells express a significantly greater amount of IgE antibodies. In figure B can be seen that under anti-CD40 + LPS conditions, significantly more IgG is expressed in both WT and miR29-KO B cells, with WT cells producing around double the amount IgG compared to miR29-KO cells. For the serum antibody production graph A, going from the WT to the miR29-KO, there is significantly less total Ig produced. However, data in figure C shows that miR29-KO cells produce more IgE compared to WT cells.

Figure D is an ELISpot plate under a microscope. The spots present on the photo are cells that are producing antibodies. As can be seen, the spots are really small and did not grow much. In order to improve the experiment and make the cells grow bigger, the ELISpot would require more time for the cells to start class switching.