The Ca^{2+} flux of $IgG1^+$ germinal center B cells is actively regulated to favor positive selection

Lingling Zhang, Amparo Toboso-Navasa, Arief Gunawan, Abdouramane Camara, Rebecca Newman, Pavel Tolar, Dinis Calado
The Francis Crick Institute, Immunity and Cancer Laboratory, lingling.zhang@crick.ac.uk

Abstract

Recent studies have shown that $IgG1^+$ germinal center B cells (GCBs) are preferentially positively selected compared to IgM^+ GCBs [1]. Despite this critical observation, the mechanisms underlying positive selection of the various isotypes in GC reactions remains unclear. We performed a genome scale CRISPR-Cas9 dropout screen in iGCB cells to identify transcription factors required for the survival/proliferation of $IgG1^+$ cells. We found Miz1 among the top required transcription factors. Previously we showed that Miz1 is highly expressed in positively selected GCBs [2]. We conditionally ablated Miz1 on monoclonal (SWHEL) and polyclonal BCR systems and this led to a striking impairment of $IgG1^+$ GCBs *in vivo*. This defect was not due to defective IgM to IgG1 class switch recombination. Instead, Miz1 was required to protect $IgG1^+$ GCBs from apoptosis specifically during positive selection. Using RNA and ChIP-Seq we investigated apoptosis modulators that are targets of Miz1. We found that the anti-apoptotic protein Tmbim4 was bound in its promoter by Miz1. Further, the expression of Tmbim4 was severely impaired upon Miz1 loss. We next found that Tmbim4 expression during positive selection was required to prevent exacerbation of the Ca^{2+} flux and to maintain the mitochondrial potential of $IgG1^+$ GCBs. This data demonstrates; unexpectedly, that $IgG1^+$ GCBs are highly susceptible to Ca^{2+} induced cell death and that active regulation of the Ca^{2+} flux is required for their survival. Given that IgG1 is critical to mediate antibody responses against viral pathogens this knowledge may allow the design of interventions that modulate the Ca^{2+} flux and tailor immune responses during vaccination.

1. Sundling, C., et al.. Immunity, 2021. 54(5): p. 988-1001 e5.
2. Toboso-Navasa, A., et al.. J Exp Med, 2020. 217(7)