

NEWS & VIEWS

Groundbreaking Discoveries in Clinical & Basic Science

CRISPR screening reveals mechanisms of IgE⁺ B-cell constraint

Clustered regularly interspaced short palindromic repeats, also known as CRISPR, provide a defense system against infections and have been found in almost all living forms.¹ The discovery of CRISPRs and CRISPR-associated (cas) genes is a new and innovative research tool that allows for the selection and identification of specific genes of interest.^{1,2} CRISPR library screening technology is being used to investigate genetic factors that may contribute to allergic disease pathogenesis, for example by targeting genes involved in lymphocyte development. Using CRISPR, multiple genes can be targeted rapidly and efficiently allowing for further understanding of complicated multigenic allergic diseases such as asthma.³ In this study

by Newman et al, CRISPR screening was used to evaluate different pathways involved in the differentiation and survival of immunoglobulin (Ig)E⁺ and IgG⁺ B cells (Figure 1).²⁻⁴ This study continues on the elucidation of the regulatory constraints placed on IgE⁺ B cells, limiting the presence of circulating IgE in the plasma and, therefore, regulating IgE antibody responses.

B-cell class switching requires the two-step process of DNA excision and ligation, with the help of specific cytokines, that leads to all Ig isotypes except for IgM and IgD. Once the B-cell class successfully switches with the help of IL-4 and the membrane-bound IgE B-cell receptor (BCR) is present, antigen-independent

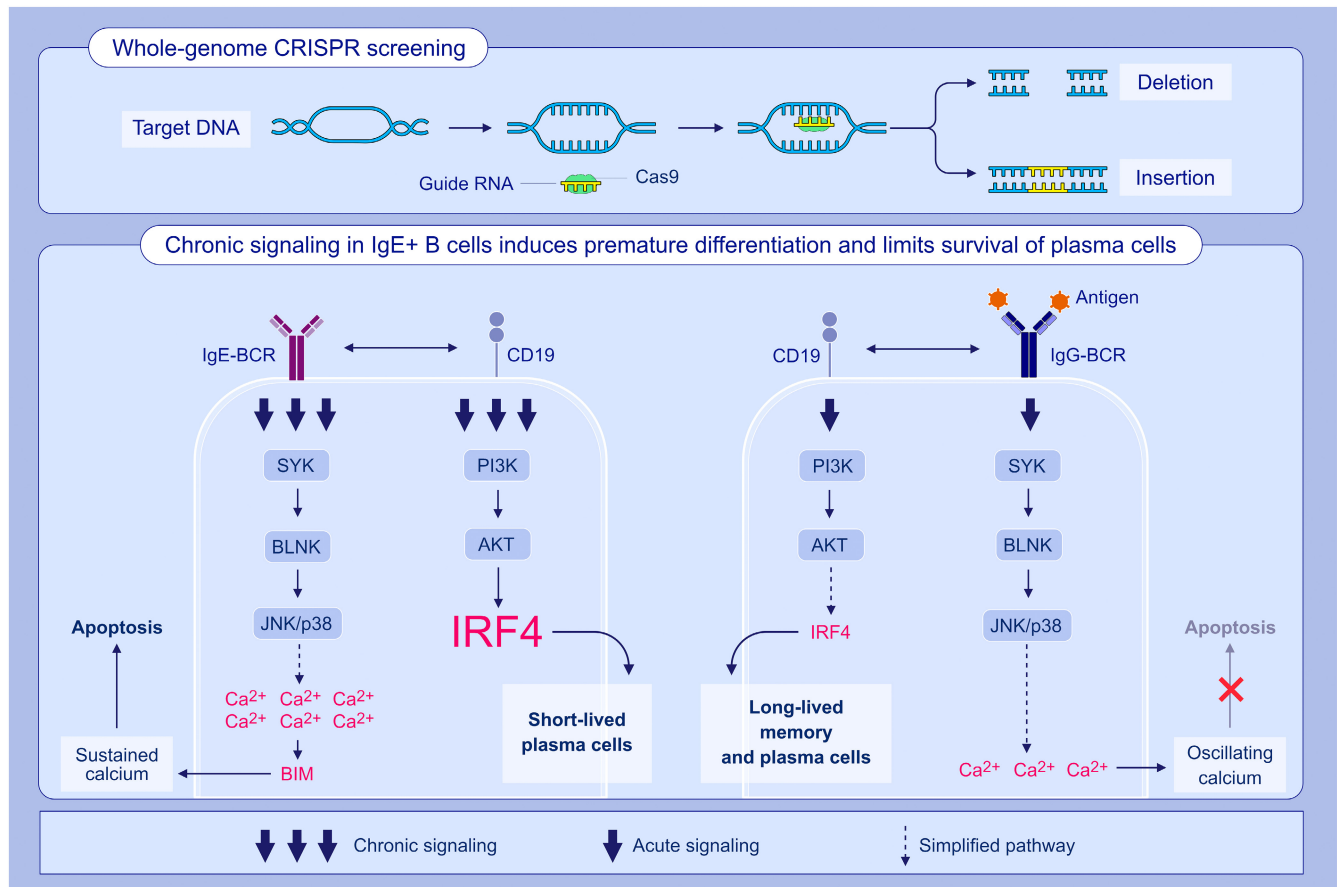


FIGURE 1 Using the novel CRISPR library screening technology, Newman et al. showed that antigen-independent IgE BCR signaling resulted in increased PI3K/mTOR signaling leading to higher IRF4 protein expression compared to antigen-dependent IgG BCR signaling. Moreover, IgE⁺ B cells demonstrated higher, chronic calcium signaling which promoted apoptosis, while IgG⁺ B cells displayed an oscillating calcium influx pattern which did not result in the required cytosolic calcium concentrations to induce apoptosis. These signaling events reveal an IgE⁺ B-cell-specific mechanism of controlling the differentiation and accumulation of IgE-producing B cells

Abbreviations: BCR, B-cell receptor; cas, CRISPR-associated; CRISPR, clustered regularly interspaced short palindromic repeats; Ig, immunoglobulin; PC, plasma cell.

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weak autonomous signaling drives premature short-lived plasma cell (PC) differentiation of IgE⁺ B cells rather than long-lived PC differentiation, thereby constraining IgE production (Figure 1).^{4,5} The IgE BCR chronically signals through the cytoplasmic adaptor BLNK (BLNK-Jnk-p38 axis) and also induces phosphorylation of the membrane adapter CD19 (CD19-PI3K-Akt-IRF4 axis).⁶ The CD19-PI3K-Akt-IRF4 axis has been known to be utilized by the IgE BCR for PC differentiation.⁶ In the study by Newman et al.,⁴ it was validated by CRISPR that IgE⁺ PCs rely on PI3K-mTOR which leads to a post-transcriptional accumulation of the transcription factor IRF4 (Figure 1). As a conclusive confirmation, the mTOR inhibitor rapamycin decreased IgE⁺ PC differentiation and IRF4 protein levels but not *Irf4* mRNA transcript levels.⁴

In addition, Newman et al.⁴ demonstrated that Syk-BLNK-Jnk-p38 induced calcium flux downstream of the BCR is a mediator of PC differentiation; however, specifically in IgE⁺ PC, chronic calcium influx leads to apoptosis (Figure 1). Calcium signaling is a well-established regulator of cellular functions, including downstream of the BCR. One way calcium signaling regulates cellular function is through apoptosis, which was first discovered in B cells in 1993 by Murphy and colleagues.⁷ More recently, calcium influx was demonstrated to be required for naive B-cell proliferation, differentiation, and survival through the NFAT and PI3K-mTOR pathway.⁸ By silencing of *Ppp3r1* (calcineurin subunit B1) via CRISPR sgRNA, Newman et al illustrated that at a later stage of B-cell maturation, calcium signaling downstream the BCR through the calcineurin-NFAT pathway downregulates PC differentiation, with a predilection in IgE⁺ B cells.⁴ They show that BCR-driven calcium release induces apoptosis through BCL2L11 (BIM) specifically in IgE⁺ germinal center B cells and PCs (Figure 1).⁴ Silencing of a calcium transporter, *Atp2b1*, led to apoptosis specifically in IgE⁺ PC's despite an equal induction of intracellular calcium in IgE⁺ and IgG⁺ PC's. Moreover, chemical inhibition of another calcium transporter, *Atp2a2*, led to reduced proportions of IgE⁺ iGC and IgE⁺ PCs, with no significant changes in IgG⁺ cells, thus convincingly demonstrating a specific role for calcium in IgE⁺ PC formation. Authors further revealed that IgE⁺ cells are more responsive to calcium-induced apoptosis due to baseline chronic calcium influx in IgE⁺ B cells (Figure 1).⁴ Conversely, IgG⁺ B cells exhibit an oscillating calcium influx pattern with reduced calcium at baseline (Figure 1).⁴ Thus, elevated chronic calcium drives apoptosis of IgE⁺ B cells most likely through BCL211. Although these in vitro data show a specific role for calcium signaling in IgE⁺ PC development compared to IgG⁺ PC's, in vivo data in an allergy model would elevate the significance of this work.

Taken together, weak antigen-independent, cell-intrinsic signaling drives IgE⁺ B-cell differentiation into PCs which is then impeded by chronic calcium influx, ultimately leading to apoptosis (Figure 1). These mechanisms delineate how long-term murine IgE⁺ B cells are restricted, thus constraining IgE-mediated responses, responses to short-lived extracellular parasite expulsion and limiting long-lived

detrimental allergic disorders. It is yet to be determined whether these specific mechanisms can be translated to human IgE⁺ B cells.

KEYWORDS

B cell, CRISPR, differentiation, IgE, plasma cell

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CONFLICT OF INTEREST

The authors declare that they have no potential conflict of interest.

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