RESEARCH HIGHLIGHT

B-cell receptor signal strength and zinc signaling: unraveling the role of zinc transporter ZIP10 in humoral immunity

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The humoral immune response, alongside cell-mediated immunity, in which B cells play crucial roles, form the primary arms of the adaptive immune system. Resting mature follicular (FO) B cells in the spleen are essential for antibody-mediated immune responses. They recirculate through the blood, and are activated upon the binding of various diverse cognate antigens to the specific B cell antigen receptor (BCR) on their cell surface. With the help of T cells, the activated FO B cells undergo the germinal center (GC) reaction, which involves massive expansion and immunoglobulin (Ig) class-switch recombination (e.g. IgM to IgG1) to elicit a high-affinity antibody response against the antigens. Zinc (Zn) is essential in immunity, and in both humans and rodents, aberrant Zn homeostasis strongly disrupts the cellularity and functions of immune cells, leading to thymic and splenic atrophy, lymphopenia, and weakened cellular and humoral immunity, which increases the host's susceptibility to various pathogens. Zn, which is transported by specific members of the Zn-transporter families, SLC39/ZIP and SLC30/ZnT, selectively fine-tunes distinct intracellular signaling events by targeting signaling molecules involved in development, growth, and immunity. Zn controls a wide range of immune signaling cascades that lead to cytokine production, antigen presentation, and the activation of kinases and transcription factors in immune cells, and disrupting the specific Zn transporter-Zn signal axis impairs cellular function. However, how Zn controls immune function, particularly, the humoral immune response, is poorly understood. In this research highlight, we review our recent finding that ZIP10-Zn signaling is required in B-cell receptor signaling for the antibody-mediated immune response.

Keywords: Zinc signaling; B cell, humoral immunity; germinal center; antigen-presenting cell

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Abrogation of antibody-mediated immune response in the absence of ZIP10

We first investigated the physiological function of ZIP10, a member of the Zn transporter SLC39/ZIP family [1], in humoral immune responses, which require the activity of antigen-presenting cell (APC) such as dendritic cell (DC), B cell, and monocyte (MO)/macrophage (Mϕ). We generated *Zip10*-conditional knockout (*Zip10*-cKO) mice, in which the *Zip10* gene was conditionally ablated under the *invariant chain* promoter (constitutively activated in APCs), and evaluated this strain's immune response ability [2]. When immunized with 4-hydroxy-3-nitrophenylacetyl conjugated to chicken gamma globulin (NP-CGG), the *Zip10*-cKO mice

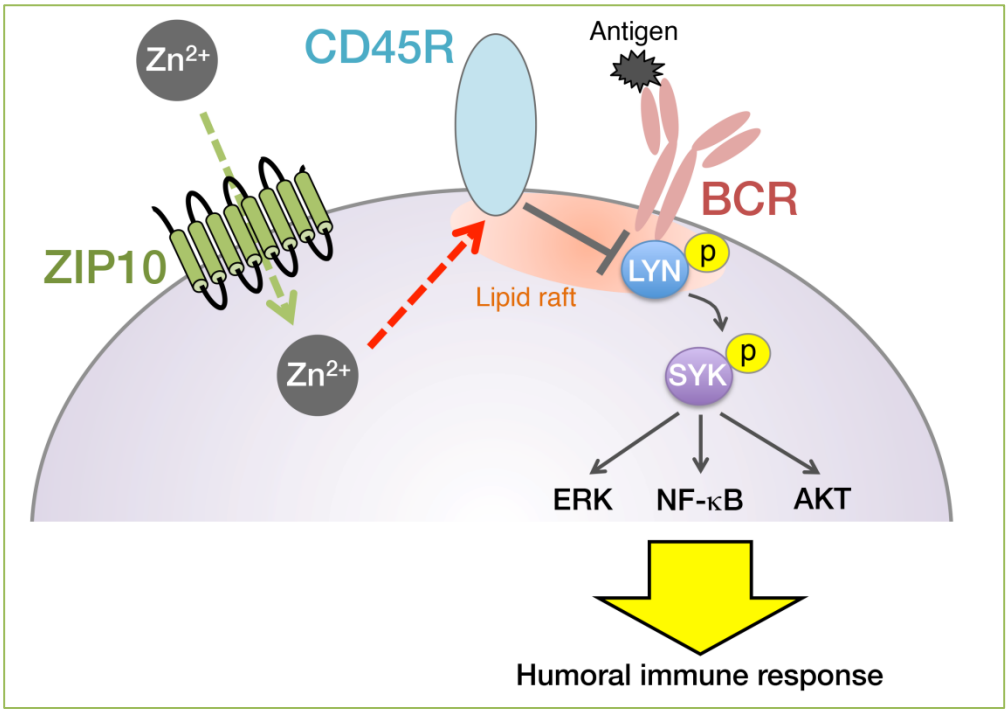

Figure 1. Model of ZIP10's role in the humoral immune response. ZIP10-Zn signaling works as a rheostat that sets the threshold for BCR-signaling strength by modulating CD45R PTPase activity. Thus, ZIP10 controls GC formation followed by the antibody-mediated immune response.

exhibited a dramatic decrease in NP-specific IgM and IgG1 production, in a B-cell-intrinsic manner. Notably, the IgG1 secretion was remarkably impaired. Successful high-affinity antibody responses require cognate T-cell help for B cells, followed by GC reactions with class-switch recombination [3]. We found that the GC ($CD45R^{+}IgD^{lo}CD95^{+}GL\text{-}7^{+}$) B cell formation was severely decreased in *Zip10*-cKO mice, although the capacity for class-switch recombination was indistinguishable from wild-type. Similar defects were also observed in Zn-deficient (ZnD) mice, confirming Zn's critical role in GC formation and thus in proper antibody responses [2].

Dysregulation of BCR signaling in the absence of ZIP10

Signaling via BCR allows B cells to be fully activated in the effector phase, and is responsible for GC formation through cognate interaction with helper T cells [4]. We were therefore interested in the activation status of BCR signaling in *Zip10*-cKO B cells. Consistent with the impaired GC formation observed *in vivo*, the proliferation of *Zip10*-cKO B cells was severely diminished after BCR cross-linking *in vitro*, but was unchanged when the cells were stimulated with toll-like receptor (TLR) ligands such as LPS (for TLR4) and CpG (for TLR9). BCR signaling induces receptor oligomerization and phosphorylation of the immunoreceptor tyrosine-based activation motifs (ITAMs) within Igα and Igβ by the SRC-family protein tyrosine kinase LYN; this recruits and activates SYK, in turn initiating the downstream activation of kinases and transcription factors such as MAPK, PI3K, and NF-κB, which are involved in cell proliferation and survival [4, 5]. Unexpectedly, *Zip10*-cKO B cells showed ERK, AKT, and NF-κB hyperactivation after BCR cross-linking, in striking contrast with their defective proliferation. We also found that BCR ligation augmented the upstream SYK and LYN activations. CD45R, which is encoded by the *Ptprc* gene, is a key receptor-type protein tyrosine phosphatase (PTPase) that is associated with lipid rafts and inhibits LYN activity [6]. We found that the overall CD45R PTPase activity in *Zip10*-cKO B cells was nearly half that in control cells. Furthermore, in *Zip10*-cKO B cells, the forced introduction of Zn suppressed the BCR-induced LYN activation by upregulating the CD45R PTPase activity. Since ZIP10 is located on the cell membrane and contributes to the uptake of Zn from extracellular fluid, our findings suggest that the ZIP10-Zn signaling axis positively and spatiotemporally controls CD45R PTPase activity through the uptake of Zn [2].

Regulation of B-cell maintenance and survival by ZIP10

We considered the physiological significance of ZIP10 in other cellular activities. Although both heterozygous and homozygous littermates in *Zip10*-cKO mice appeared healthy and were fertile under specific-pathogen-free conditions, the effect of APC-specific *Zip10* ablation was discernable in a cell-autonomous decrease in FO B cells [2]. In addition, when the ZIP10 gene was selectively ablated at an early B-cell

developmental stage under the *Mb-1* (Igα gene) promoter, pro-B cell survival in the bone marrow was severely decreased [7]. In fact, the inducible deletion of ZIP10 in pro-B cells reduced the intracellular Zn, and spontaneously increased the caspase activity, resulting in cell death. Chemically chelating the intracellular Zn mimicked this phenomenon. Moreover, we demonstrated that JAK-STAT3/STAT5 activation positively regulated the ZIP10 expression, and that the resultant ZIP10-Zn signaling axis controlled early B-cell survival by inhibiting caspase activity [7]. Thus, our results underscore a definitive role for ZIP10 in survival and maintenance in both early and mature B cell populations.

Conclusions

Our understanding of Zn's essential role in immune systems, both *in vitro* and *in vivo*, has grown rapidly in recent decades through Zn-deficiency studies in rodents and humans [8-17]. Our recent study demonstrated ZIP10's physiological importance in B-cell-mediated humoral immune responses, increasing our understanding of how Zn exerts its effects on immune function. We found that a lack of ZIP10 led to dysregulated BCR signaling due to reduced CD45R PTPase activity, and impaired cell proliferation, thus, the IgG1 antibody response following GC formation decreased markedly in a ZIP10-deficient environment. Therefore, ZIP10 functions as a rheostat that modulates BCR signal strength by setting the threshold. In consequence, ZIP10 controls humoral immunity by regulating the full antibody responses mounted through effective GC formation **(Fig. 1)**. Our study provides a possible mechanism that addresses the long-standing question of how Zn controls lymphocyte functions and homeostasis. Indeed, Zn signals are involved in various physio-pathological phenomena [14-23], so that further exploration of ZIP10-Zn signaling in other receptor-mediated signaling pathways will shed more light on the relevance of Zn signals in general health and disease state.

Conflict of Interests

We declare there is no potential conflict of interests.

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Author contributions

S.H. has made contributions to design, drafting the manuscript, and revising it. T.M. has made contributions to drafting and revising the manuscript. T.F. has made design, drafting the manuscript, revising it, and has given final approval of the version to be published.

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