



Letter to the Editor

Advantages of targeting B cell receptor complex to treat B-cell derived autoimmune diseases and lymphomas



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ABSTRACT

Antibodies produced by B-cells provide protection from infectious agents. However, impaired cell death signaling pathways in B-cells can lead to cancer, immunodeficiency or autoimmune diseases. B-cell signaling molecules such as CD20, CD19, Btk, and BAFF-R are targeted by therapeutic drugs and used to treat B-cell derived lymphomas or autoimmune diseases. Nevertheless, B-cells could develop resistance to these therapeutic drugs or the therapeutic drugs may have off-target effects. For instance, repeated rituximab (anti-CD20 antibody) treatment may lead to the loss of its target cell surface molecule, CD20. In addition, in B-cell malignancies, loss of CD19 expression has been observed. Another target molecule, Btk is expressed not only in B-cells but also in mast cells, macrophages, and dendritic cells. Thus, targeting Btk could negatively regulate the functions of innate immunity. The expression of BAFF-R is thought to be restricted to B-cells but it is also expressed on T-cells. Targeting BAFF-R, therefore, may lead to depletion of T-cells in addition to B-cells. B cell receptor (BCR) expression and signaling, however, are critically important for development, differentiation and survival of B-cells. Moreover, BCR is exclusively expressed on B-cells, which makes it an excellent target to avoid off-target effects.

B-lymphocytes (B-cells) are core components of adaptive immunity. The main functions of B-cells include antigen presentation and antibody production. However, defects in B-cell growth, development and selection can result in malignancy, immunodeficiency, and autoimmunity (Yanaba et al., 2008; Basso and Dalla-Favera, 2015; Ahn and Cunningham-Rundles, 2009). B cell receptor (BCR) is a transmembrane receptor protein located on the surface of B-cells. Signaling through BCR is required for normal progression of B-cell development. In humans, B-cells are produced in the bone marrow from hematopoietic precursor cells and they migrate to secondary lymphoid organs (periphery) where they encounter antigens. In the bone marrow, the VDJ recombination process produces BCR repertoires that are able to recognize a large array of antigens. In the periphery, B-cells can be activated by a variety of infectious agents through BCR. This phenomenon leads to the formation of germinal centers (GCs) (Pieper et al., 2013). In GCs, B-cells undergo somatic hypermutation (SHM), which increases BCR diversity and thereby increases chances of producing B-cells with high affinity to antigens. However, the SHM process can also lead to the production of autoreactive- B cells (Mackay and Rose, 2001).

The BCR complex consists of an antigen-binding subunit and a signaling subunit. The antigen binding subunit is a membrane-bound immunoglobulin (mIg) which lacks any signaling motif. The mIg part consists of two heavy and two light chains combined to each other by disulfide bonds. The signaling subunit consists of the accessory proteins CD79a (Ig α) and CD79b (Ig β) which transmit the activating signals to the cells interior. The recognition of specific antigens by the mIg, therefore, leads to the activation of a number of kinases such as Brutońs tyrosine kinase (Btk), phosphatidylinositol-3-kinase (PI3K) and extracellular signal-regulated kinases (ERKs) (Niiron and Clark, 2002; Treanor, 2012).

As we demonstrate in our previous work, the early activation of ERK1 and ERK2 following BCR stimulation results in short-term survival of GC B-cells. However, in the late phase, BCR stimulation leads to inhibition of ERK1 and ERK2, which correlates with cell death (Adem et al., 2015, Fig. 1). Interestingly, BCR-mediated apoptosis of GC B-cells is reversed by CD40 signaling (Adem et al., 2015, Fig. 4). These findings indicate that B-cells which overexpress ERK1 and ERK2 might bypass the selection process. Thus, B-cells which overexpress ERK1/2 and possess memory or plasma cell transcription signatures may transform into self-reactive memory B-cells or auto-antibody producing plasma cells. In addition, it is also possible that B cells which overexpress ERK1/2 but lack differentiation specific signatures may transform into lymphoma cells. Overexpression of Rasgrf-1 amplifies the Ras-ERK pathway in chronic lymphocytic leukemia cells, thereby enhancing B-cell survival. This indicates that malignant B-cells utilize ERKs for their survival (Liao et al., 2014). Moreover, changes in the activation of Ras-ERKs pathway may lead to autoimmune manifestations (Teodorovic et al., 2014). Thus, targeting ERKs may have therapeutic benefits against these B-cell derived diseases. However, because of the critical role of ERKs in various cellular functions, the use of ERK inhibitors can lead to severe cellular toxicity. It is, therefore, imperative to selectively target B-cells in order to avoid the side-effects which could result from off-target inhibition.

Rituximab is a chimeric monoclonal antibody which targets CD20, the cell surface differentiation antigen on B-cells. CD20 has a role in B-cell activation and differentiation. It is expressed in mature B-cells but not in hematological stem cells, pro-B cells, normal plasma cells or other tissues. This unique characteristic of CD20 expression makes it the suitable target to treat B-cell derived lymphomas or autoimmune diseases (Du et al., 2007). However, rituximab does not deplete autoreactive antibody-producing plasma cells, because these cells do not express CD20. Nevertheless, rituximab depletes B-cells, thereby decreasing the production of autoreactive antibody and malignant B-cells. The loss of CD20 expression, however, after repeated rituximab treatment may contribute to B-cells' resistance against rituximab (Haidar et al., 2003). A study on CD20^{-/-} (knockout) mouse has shown normal development and function in CD20 deficient B-cells (Uchida et al., 2004). This data further strengthen the fact that CD20 is not a critical cell surface molecule for the survival and development of B cells. CD19 is another pan B-cell marker used to target and deplete B-cell

populations. However, different types of lymphomas lose CD19 expression (Masir et al., 2006).

Targeting signaling molecules or their receptors which have critical functions in B-cell development or survival helps to overcome drug resistance which results from the lack of CD20 or CD19 expression. As the majority of mature B-cell lymphoma express functional BCR, several small molecule inhibitors have been developed to inhibit a variety of kinases in the BCR pathway including Btk (Rickert, 2013). However, Btk is also expressed in several other hematopoietic lineages such as neutrophils, thrombocytes, mast cells, macrophages and dendritic cells (Brunner et al., 2005; Honda et al., 2012; Futatani et al., 2001). Inhibition of Btk, therefore, could compromise innate immunity. In this regard, the adverse events of treating chronic lymphocytic leukemia/lymphomas patients with Ibrutinib, a BTK inhibitor, include infections, neutropenia, and thrombocytopenia (Varma et al., 2016). Moreover, the lack of tissue specificity of Ibrutinib limits their utility. However, lymphoma patients respond to Ibrutinib.

BAFF-R is another surface molecule used to target B-cells and its expression is thought to be B-cell specific. Interestingly, BAFF-R is also expressed in T-cells subsets (Ng et al., 2004). The elimination of B-cells through BAFF-R could also deplete T-cells populations, thereby negatively regulate T-cell-mediated immunity. Several studies have demonstrated the efficacy and safety of anti-BAFF agents such as tabalumab and atacicept. The data show considerable variability in response to BAFF-R/BAFF blockage. For instance, tabalumab did not provide the intended clinical efficacy in rheumatoid arthritis (Smolen et al., 2015) and multiple myeloma patients (Raje et al., 2017). Moreover, treatment with atacicept was well tolerated and no dose-limiting toxicity was observed in B-cell lymphoma patients, however, none of the subjects achieved complete or partial responses (Ansell et al., 2008). These poor clinical responses may be due to the substantial heterogeneity in the pathogenesis of autoimmune diseases/lymphomas.

Given the complexity of the pathogenesis of B-cell derived diseases, it is imperative to identify a potential target molecule that is B-cell specific and critical to cell survival. In this regard, BCR could be an excellent target to eliminate a malignant or autoreactive B-cells because BCR is required for the development and survival of B-cells. In $\lambda 5$ knockout mice, B-cell development was severely impaired (Miyazaki et al., 1999). In addition, conditional deletion of surface IgM in mature B-cell leads to rapid apoptosis, indicating that regardless of antigen encounter, signaling through BCR is necessary for the survival of B-cells (Lam et al., 1997). Therefore, it is less likely that BCR-dependent lymphomas/auto-reactive B-cells develop resistance by losing BCR expression because the loss of BCR compromises B-cell survival. As shown in mice study, inhibition of B cells through CD79b is much more effective than depletion of B-cells with the anti-CD20 antibody (Bruhl et al., 2015). In addition, anti-CD79b antibody induces B-cell anergy via downregulation of BCR components. This distinct feature of the anti-CD79b antibody shows that it also suppress the pathogenic immune responses in the absence of B cell depletion (Hardy et al., 2014). Furthermore, a clinical trial study shows that patients diagnosed with relapsed or refractory B-cell lymphoma responded to polatuzumab vedotin (an anti-CD79b drug conjugate) (Pfeifer et al., 2015), suggesting that BCR complex could be the suitable target to treat lymphomas.

Interestingly, the expression of CD79a and CD79b is restricted to B-cells and these co-receptors are not expressed in other healthy tissue and immune cells. Nonetheless, CD79a is found to be expressed in T-cell lymphoma (Chu and Arber, 2001; Hashimoto et al., 2002). Moreover, there is a case report indicating the co-existence of B-cell and T-cell lymphoma in some patients (Campidelli et al., 2007). Thus, the expression of CD79a in T-cell lymphoma in addition to B-cell lymphoma could be exploited as the therapeutic target to treat B-cell and T-cell lymphoma simultaneously, however, the dual presence of these diseases in a single patient is an exception.

Epstein-Barr Virus (EBV)-derived lymphoma cells may not retain dependency on BCR for their survival. Because EBV infection can rescue BCR⁻ (B-cell with no BCR expression) GC B cells from apoptosis (Mancao et al., 2005). Therefore, this finding indicates that targeting BCR complex could also have its limitation and should be considered according to the origin of the disease.

The mIg could also be used as a therapeutic target, for example, monoclonal antibody targeting epitope restricted to mIgM has anti-tumor effects (Welt et al., 2016). The anti-tumor effects of the anti-mIgM antibody, however, could not be extended to follicular lymphoma (FL), because FL originates from B-cells which have undergone class-switching to IgG (Aarts et al., 2002). Moreover, the anti-IgD antibody is efficient in depleting mature B cells (Nguyen et al., 2010). However, IgD might not be the best target to eliminate self-reactive memory B cells because these cells lack IgD expression. Idiopes (Ids) are other mIg components that have been used as therapeutic targets against cancer (de Cerio et al., 2007). The limitations with this approach are the therapeutic antibodies needed to be individually customized and the hypervariable region associated Ids may be lost due to residual hypermutation activity in target B-cells.

Altogether, these data show that the BCR complex, either the mIg (constant domain) or CD79a/CD79b is the suitable therapeutic target for B-cell derived diseases. It might also be used as a target for patients who do not respond to B-cell specific immunotherapy such as anti-CD20 or anti-CD19 antibody. Thus, the anti-BCR antibody can be used as a cell-specific therapeutic agent against autoimmune diseases and lymphomas. Furthermore, we suggest that the anti-BCR antibody conjugated with a plant or bacterial-derived toxin could enhance therapeutic efficiency.

Conflict of interest statement

The authors declare no financial or commercial conflict of interest.

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