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Review

The mechanistic impact of CD22 engagement with epratuzumab on B cell function: Implications for the treatment of systemic lupus erythematosus

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ABSTRACT

Epratuzumab is a B-cell-directed non-depleting monoclonal antibody that targets CD22. It is currently being evaluated in two phase 3 clinical trials in patients with systemic lupus erythematosus (SLE), a disease associated with abnormalities in B-cell function and activation. The mechanism of action of epratuzumab involves perturbation of the B-cell receptor (BCR) signalling complex and intensification of the normal inhibitory role of CD22 on the BCR, leading to reduced signalling and diminished activation of B cells. Such effects may result from down-modulation of CD22 upon binding by epratuzumab, as well as decreased expression of other proteins involved in amplifying BCR signalling capability, notably CD19. The net result is blunting the capacity of antigen engagement to induce B-cell activation. The functional consequences of epratuzumab binding to CD22 include diminished B-cell proliferation, effects on adhesion molecule expression, and B-cell migration, as well as reduced production of pro-inflammatory cytokines, such as IL-6 and TNF. Studies in patients treated with epratuzumab have revealed a number of pharmacodynamic effects that are linked to the mechanism of action (i.e., a loss of the target molecule CD22 from the B-cell surface followed by a modest reduction in peripheral B-cell numbers after prolonged therapy). Together, these data indicate that epratuzumab therapy affords a unique means to modulate BCR complex expression and signalling.

Contents

1. Introduction

SLE is an autoimmune disease associated with dysregulation of both innate and adaptive immune functions, including polyclonal B-cell

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activation, autoantibody production, hypergammaglobulinemia, and immune complex formation, ultimately leading to end-organ damage and dysfunction [1–3]. There is a clear medical need for new treatments of SLE and, indeed, a monoclonal antibody targeting a B-cell survival factor (belimumab) was approved recently for SLE following successful phase 3 clinical trials [4,5]. However, the anti-CD20 B-cell-depleting monoclonal antibodies, rituximab and ocrelizumab, and agents targeting the BAFF/APRIL axis, tabalumab and atacicept, did not meet clinical end-points in randomized clinical trials [6–11], suggesting that unique characteristics of a B-cell targeting agent might be required for successful treatment of patients with SLE. Epratuzumab is a humanised monoclonal antibody that targets CD22 on B cells and was initially developed for, and shown to be effective in, patients with B-cell malignancies [12–14], where it was first recognized that normal B cells in such patients did not show more than a 50% reduction in peripheral blood. It is also currently being evaluated in two phase 3 clinical trials in patients with SLE (NCT01261793 & NCT01262365). In the earlier phase 2b study, and its open label extension, epratuzumab produced sustained improvements in disease activity in patients with active SLE [15–17]. Thus, understanding the impact of epratuzumab on B-cell function should provide insights into the specific actions of B cells involved in SLE pathogenesis and rational approaches to target and modulate them effectively.

2. B cells and B-cell receptor (BCR) activation play an important role in SLE

The potential to secrete antibodies is often considered to be the major function of B cells, but these cells perform many other functions: they can serve as antigen-presenting cells, produce cytokines, chemokines and other mediators, regulate the activity and function of neighbouring cells (notably T cells and dendritic cells), and may serve suppressive (tolerance) functions [18–22]. The most unique feature of B cells is the expression by each B cell of a unique receptor (the B-cell receptor (BCR)) derived from recombination of distinct genetic components. The BCR not only conveys a specific antigen-binding capability to each B cell, but also plays a critical role in B-cell development, activation and survival, thus regulating the ultimate fate of B cells [23,24].

In the context of SLE, there is considerable evidence for a disturbed homeostasis of peripheral B cells [25–27]. For example, there is a large increase of antigen-experienced $CD27^{+}/IgD^{-}$ post-switched memory B cells [28], possibly indicative of a loss of peripheral tolerance. Additionally, expanded numbers of $CD27^{-}/IgD^{-}$ ("double negative") memory B cells have been demonstrated in active disease [29], and further refinement of this population identified CD95 (Fas) as a marker of a subset of $CD27^{-}$ memory B cells with an activated phenotype [30]. This subset of recent germinal centre emigres is elevated in SLE patients with disease flares, and their presence correlates with disease activity and serologic abnormalities. Moreover, a $CD19^{hi}$ population [31,32], as well as $CD21^{lo}$ B cell subset [33,34], were found to be characteristic of SLE and to correlate with long-term adverse outcomes. It has also been reported that a particular $CD27^{-}$ B cell subset with increased intracellular Syk (Syk^{hi}) expression also is elevated in SLE [35]. Furthermore, active SLE is characterised by expanded populations of $CD27^{hi}$ plasma cells and plasmablasts, including $HLA\text{-}DR^{hi}/CD27^{hi}$ cells [36] that represent newly formed and recently divided plasma cells. The presence of these Ig-secreting plasma cells correlates with lupus activity and anti-dsDNA antibody titers. Interestingly, a recent study identified a distinct subset of activated naïve (acN) B cells as a source of antibody-secreting cells and autoantibody production during SLE flares [37]. Finally, B cells with a regulatory phenotype (so-called 'Bregs') appear to be defective in SLE. Whereas $CD24^{hi}/CD38^{hi}$ Bregs from healthy individuals can inhibit the differentiation of Th1 cells and release IL-10, the same population isolated form SLE patients lacked this capacity [38]. Overall, it appears that B cells are involved in a positive feed-forward loop that maintains autoimmune memory and immunopathology in SLE [39], and may lack appropriate regulatory elements.

In addition to phenotypic abnormalities, there is also evidence for hyper-reactivity of B cells in SLE [40,41]. For instance, B cells from SLE patients display augmented signalling responses, including increased tyrosine phosphorylation and calcium flux both spontaneously [42] and in response to BCR ligation [43–45], compared to healthy control B cells. In fact, recent data demonstrated that the hyper-responsive phenotype of SLE B cells (enhanced Syk phosphorylation) can be induced in healthy B cells after incubation with SLE plasma [46]. A broad kinome array analysis revealed changes in the activation of a number of key kinases in SLE B cells, such as increased phosphorylation of phosphoinositide 3-kinase and Akt-1 (PKB), as well as abnormal phosphatase activity, indicated by decreased SHIP phosphorylation [47]. SLE B cells also show constitutively enhanced expression of co-stimulatory molecules, such as CD40L, defective signalling through FcγRIIb, enhanced cytokine production, spontaneous Ig class-switching and production, and possess markedly enhanced mutational activity in their Ig gene repertoire [41,48–51]. However, B cells from patients with severe disease have been shown to be hypo-responsive in relation to cytokine production after toll-like receptor (TLR) stimulation [52], although they are hyper-proliferative in response to combined stimulation through BCR/CD40 or BCR/TLR pathways [53]. Interestingly, in spite of an overall reduced level of Lyn, the lipid rafts of B cells from SLE patients are large and intensely stained, and contain increased levels of phosphorylated Lyn, suggestive of an enhanced activation state [54] or possibly enhanced 'tonic' (ligand-independent) signalling. Finally, both SHP-1 and CD45 are down-regulated in some patient B cells [55], possibly indicating that compensatory pathways are operative in disease. By contrast, there is low activity of PTEN, a phosphatase that serves to down-regulate BCR downstream signals, in SLE B cells [45]. Overall, the data strongly suggest that BCR-related pathways are aberrantly up-regulated in SLE B cells. This is in line with the results of genome-wide association studies which linked B cell signalling genes, such as *PTPN22*, *BANK1* and *BLK*, with SLE [56–58]; very recent data also identified an SLE risk allele in the *PXK* gene which encodes a protein shown to be involved in BCR internalisation [59].

3. The principal role of CD22 is to regulate the BCR

CD22 is a membrane receptor found on B cells and is a member of the Siglec family of proteins (designated Siglec-2). The Siglec family members are mainly expressed on immune cells, are homologous in sequence and also share structural features, most notably the presence of Ig-like extracellular domains and the capacity to recognize sialic acid-containing ligands [60,61]. CD22 primarily exists as a protein containing 7 Ig-like domains, the most membrane-distal of which (domain 1) is responsible for ligand binding. The expression of CD22 is generally regarded as being restricted to B cells. CD22 is initially expressed on immature B cells, is present throughout most of B-cell development, being most highly expressed on naïve B cells [62], but is lost on plasmablasts and plasma cells. Nevertheless, memory B cells and germinal centre B cells from human tonsils express CD22 to a significant degree [63].

Binding of antigen to the BCR complex triggers a cascade of phosphorylation events and changes in calcium (Ca^{2+}) flux that ultimately drive B-cell activation (reviewed in [64]). Upon BCR cross-linking, Lyn phosphorylates the immunoreceptor tyrosine-based inhibitory motifs (ITIMs) on CD79α/β which creates docking sites for other protein tyrosine kinases, such as spleen tyrosine kinase (Syk), which in turn phosphorylate and recruit the adaptor molecule, BLNK/SLP65 (B-cell linker protein). BLNK/SLP65 forms a scaffold for the association of numerous signalling components, including Vav-1, Btk (Bruton's tyrosine kinase), and phospholipase Cγ2 (PLCγ2). Activation of the inositol triphosphate/diacylglycerol pathway by PLCγ2 promotes Ca^{2+} release from intracellular stores, which in turn triggers an influx of calcium through the opening of Ca^{2+} release-activated channels (CRACs), and

activation of NFκB, NFAT and ERK signalling pathways. Ca^{2+} efflux can also be regulated via plasma membrane Ca^{2+} ATPase (PMCA4).

Based on a wealth of published data, CD22 is believed to play a primary role as an inhibitory co-receptor of the BCR. In this manner, BCR-activation initiates phosphorylation of key tyrosine residues on the intracellular region of CD22 by Lyn and other Src family members. Once phosphorylated, such tyrosine residues become docking sites for Src homology (SH2) domain-containing proteins, such as SHP-1, ultimately leading to de-phosphorylation of BCR signalling components and a reduction in Ca^{2+} flux in B cells [65,66]. By this mechanism, CD22 serves to control the threshold of the BCR response. Although BCR-related research has focused on antigen-driven events and their importance to immune function, ligand-independent (or 'tonic') signalling also has been described and appears to play a key role in normal B-cell development, maturation and survival [67–69]. However, the role that CD22 plays in modulating 'tonic' BCR signals has not been investigated.

It is well accepted that combinatorial signalling through BCR and TLR pathways shapes the outcome of B-cell activation [70] and, in general, simultaneous BCR cross-linking synergistically enhances TLR responses and vice versa [71–73]. Other work has indicated that there is cross-talk between BCR and CD40/CD40L pathways [74,75], and CD40L activation can influence the phosphorylation of CD22 [76]. Moreover, data from CD22-deficient mice argue that CD22 negatively regulates TLR signals in a constitutive manner [77,78] and CD40 stimulation is able to rescue B-cell apoptosis and induce enhanced proliferation of B cells from CD22-deficient mice [79,80], suggesting that the regulatory function of CD22 may be exerted beyond the BCR pathway per se.

In resting B cells, CD22 and BCR proteins are distributed homogenously on the B-cell surface and probably in physically separate microdomains of the cell membrane. Upon ligation, the BCR migrates into distinct areas in the membrane where it is eventually internalised. This process is important for the B cell to take up BCR-bound antigen for processing and eventual presentation to T cells. After binding cognate antigen, the BCR migrates to clathrin-coated pits and lipid rafts, where clathrin is also located [81,82]. In resting B cells, CD22 is also normally excluded from lipid rafts, but may reside in other clathrin-rich regions of the plasma membrane [83]. Following BCR ligation, however, CD22 migrates into the same vicinity as the BCR, although there is a debate as to whether this occurs within lipid rafts or other domains [76, 84–86]. It appears that the BCR may be constitutively in association in some B-cell lines and tonsil B cells [87,88].

The ligand for CD22, α2,6-sialic acid, decorates a large number of cell surface and soluble proteins, including CD45, IgM and CD22 itself. Ligands can be presented to CD22 in *cis* (on the same cell) or in *trans* (on opposing cells or on soluble proteins). Interactions in *trans* have been postulated to regulate B-cell adhesion events and may also be important for recognition of 'self' [22,89]. However, in terms of CD22 regulation of the BCR, the consensus view is that *cis* ligand interactions play a predominant role, although two seemingly conflicting models have been proposed [90]. In the first, *cis* interactions increase CD22-BCR association because several BCR-related proteins express high levels of CD22 ligand, whereas in the second model, *cis* interactions with non-BCR-related proteins, such as CD22 itself, result in reduced association with the BCR. Both models may be correct in different contexts. It is important to note that epratuzumab does not bind to the ligand-binding domain 1 of CD22 and does not interfere with ligand binding and therefore would not interfere directly with either mechanism. CD22 can also interact with neighbouring cell surface proteins in *cis* through covalent protein–protein interactions, independent of the presence of the α2,6-sialic acid ligand [91].

In a broader context, CD22 is considered to be one of several B-cell surface receptors that have been called "response regulators" and which are responsible for fine-tuning BCR signals [92,93]. Other "response regulators" include CD19, CD72 and FcγRIIb. The "CD19/CD22 signalling loop", providing positive versus negative regulatory activity on BCR signalling, respectively [94], represents a major pathway for BCR fine-tuning, whereby the two molecules modulate the signalling competence of each other as well as of the BCR itself.

4. Epratuzumab can down-modulate BCR-driven signalling

Given the normal role that CD22 plays in regulating BCR-driven signalling, it was important to explore the impact of epratuzumab on the downstream events following BCR engagement. The regulatory activity of CD22 is initiated by CD22 phosphorylation, and immunoprecipitation data in B-cell lines demonstrated that epratuzumab can rapidly induce the direct phosphorylation of CD22 [95] as well as its migration to lipid rafts [86]. Moreover, more recent studies have shown enhanced CD22 phosphorylation in primary human B cells with epratuzumab followed by direct co-localisation of CD22 with SHP-1 (Fleischer, Lumb & Dörner, unpublished results).

Sieger et al. [96] used flow cytometry to show that epratuzumab pre-incubation with primary B cells in vitro diminished the subsequent phosphorylation of both Syk and PLCγ2, and reduced Ca^{2+} flux after BCR stimulation. Inhibitory effects were demonstrated in both $CD27^{-}$ naïve B cells and in $CD27^{+}$ memory B cells, and occurred whether epratuzumab was used as an intact IgG or as a $F(ab')_2$ fragment lacking Fc, indicating no role for Fc receptor binding. In other experiments, a direct effect of epratuzumab on the Ca^{2+} efflux pump, PMCA4, was suggested. Data supporting the potential for epratuzumab to dampen Ca^{2+} flux were also obtained in experiments using B cells from human CD22 transgenic mice [97], in which the mouse B cells express human CD22.

In a study of peripheral blood mononuclear cells from 60 healthy donors [98], epratuzumab inhibited anti-BCR-induced proximal phosphorylation events, including decreasing phospho-Syk, -PLCγ2, -Btk and -Slp76, in naïve B cells, as well as class-switched and pre-switched memory B-cell subsets. Interestingly, somewhat more distal BCR signals were modulated differentially in different B-cell subsets, such that phosphorylation of ERK and p38, for example, were inhibited in memory B-cell subsets but broadly upregulated in naïve B cells. Intriguingly, the interaction of intravenous immunoglobulin (IVIg) with CD22 on B cells has been shown to inhibit proximal BCR signals and to activate phosphorylation of p38 and ERK, thereby promoting apoptosis [99]. There was significant donor to donor variation in responses across the 60 donors, which may be relevant in relation to predicting individual patient responses.

5. Epratuzumab decreases CD22 and components of the BCR complex from the B-cell surface

Epratuzumab, like other CD22 antibodies, is known to induce the loss of its target antigen from the cell surface [95,97,100]. The process occurs rapidly in vitro (within 30–45 min), reaching a maximum of about 70–80%. The principal mechanism is internalisation into the B cell. Epratuzumab directly induces the rapid movement of CD22 into (or into the vicinity of) lipid raft domains in the absence of BCR activation [86], and likewise induces the co-localisation of CD22 with the BCR component, CD79α [96], which is then internalised along with CD22. This suggests that epratuzumab induces the removal of components of the BCR complex through an "innocent bystander" mechanism. Both the co-localisation of CD22 with the BCR and subsequent internalisation events can be induced with a $F(ab')_2$ fragment of epratuzumab, but not with a monovalent Fab, arguing that such pathways require bivalency but not the Fc domain of the antibody. Although there are no data with epratuzumab itself, studies with other anti-CD22 antibodies have shown that internalised proteins can potentially be delivered both into lysosomal compartments for degradation [101] or recycled from endosomal compartments [83,102,103], although recycling occurs at a much slower rate compared to other recycling receptors.

In addition to CD22, epratuzumab also induced the loss of a range of other proteins from the B-cell surface in vitro, including CD19, CD79β

and CD21, whilst the expression of other proteins, such as CD40, CD45 and CD18, was largely unchanged [100]. An alternative mechanism was suggested for this surface clearance, namely trogocytosis (from the Greek "to gnaw"), or the transfer of material from one cell to a neighbouring cell. Importantly, the loss of CD19 and CD22 from the B-cell surface was associated with a concomitant up-regulation of these proteins on phagocytic cells, notably monocytes. Unlike internalisation, which is an Fc-independent process, trogocytosis is dependent on interactions with Fc receptors and does not take place if an $F(ab')_2$ fragment of epratuzumab is employed. The relative contribution of internalisation and trogocytosis to the clearance of components of the BCR complex from the surface of individual B-cell subsets is currently unknown.

Overall, one can view internalisation and trogocytosis as representing different but complementary mechanisms that decrease expression of components of the BCR complex from the B-cell surface. Reduction of CD19 may be of particular relevance for SLE, because polymorphisms of CD19 have been associated with susceptibility to disease, and loss of CD19 has been shown to attenuate activation of B cells by raising the BCR signalling threshold [104,105].

6. Physical interactions between CD22 and the BCR complex are known to inhibit B-cell activation

Inducing direct interaction of CD22 with the BCR dampens B-cell activation. For instance, co-expression of α2,6-sialic acid CD22 ligands with antigen on target cells leads to diminished activation of B cells [89]. The use of synthetic antigenic polymers displaying both a T-independent antigen (dinitrophenol) and CD22 ligands that physically bring CD22 and the BCR together leads to dampened BCR signalling in vitro and reduced antibody responses in vivo [106,107], whilst antibody-induced clustering of CD22 with the BCR also leads to dampened Ca^{2+} flux [108]. By contrast, sequestration of CD22 *away from* the BCR using antibody-coated beads lowers the activation threshold and increases proliferation of human B cells [65]. Finally, liposomes presenting both antigen and CD22 ligands markedly inhibited B-cell activation events, enhanced apoptosis in vitro, and diminished antibody responses in vivo [22] and lymphocytes bearing foreign antigen as a model of donor-specific transfusion (IgM^{HEL} cells) deleted antigen-reactive B cells in vivo through BIM-dependent apoptosis [109]. This phenomenon has been termed STALing (SIGLEC-engaging tolerance inducing antigenic liposomes) [110], and was shown to be successful in preventing antibody responses in a preclinical model of haemophilia A [22].

The endocytosis of the BCR is a normal event that is important for its functional activity. Recent work [111] has shown that antigens that can induce co-clustering of the BCR and CD22 promote rapid BCR endocytosis even at low concentrations, whereas slower endocytosis occurs with antigens that bind only the BCR. This is likely to be relevant to the situation with epratuzumab, given its propensity to induce clustering of CD22 with the BCR in lipid raft domains with a high internalisation rate. Moreover, since CD22 appears to exist primarily as large homomultimeric complexes on the cell surface [112], formed because of interactions mediated by *cis* ligand interactions or via covalent homotypic binding (neither of which would be disturbed by epratuzumab), an intriguing hypothesis is that epratuzumab pulls these very large complexes into association with the BCR, maximizing the clearing of the BCR from the B-cell surface.

7. Epratuzumab exerts functional effects on B-cell activation

Physiological activation of B cells takes place in a complex microenvironment, in which B cells are likely receiving a combination of signals, depending on their location, the presence of other cells and cytokines, the nature of a given immune challenge and disease state. More recent studies investigating the mechanism of action of epratuzumab have attempted to mimic aspects of this complexity by, for example, providing combinatorial signals, assessing B cells co-cultured with other leukocytes, and comparing SLE with healthy donor B-cell responses.

B cells from the blood of patients with SLE display enhanced proliferative responses to anti-BCR activation and also to combinations of anti-BCR plus CD40L or anti-BCR plus CpG oligodeoxynucleotides (used as a TLR9 agonist) [53]. Epratuzumab in these culture systems was shown to inhibit the enhanced proliferative responses of SLE B cells, but not those from healthy donors. By contrast, other data have demonstrated that, under certain conditions, an enhancement of BCR-driven proliferation of tonsillar B cells or B-cell lines can be observed with some CD22 antibodies, although such responses typically require the antibodies to be cross-linked in solution or to be immobilised on a surface [65,113,114]. Importantly, enhanced proliferation was a feature of CD22 antibodies that could block ligand binding, whereas non-ligand blocking antibodies lacked activity [115]. Particular care is needed when comparing data with transformed B cell lines and primary B cells, given the paradoxical consequences of BCR activation which can enhance proliferation of primary mature B cells [65], but induce apoptosis of transformed B cell lines [116]. With this in mind, a recent study using epratuzumab showed that immobilisation of the antibody (coating on plates or captured onto endothelial cells) in the absence of BCR activation was able to induce apoptosis and decrease the viability of Ramos and Daudi human B-lymphoma lines [117]. Notably, the inhibition of B-cell proliferation that occurs following direct cell–cell interaction with dendritic cells has been shown to be CD22-dependent [118].

The effect of epratuzumab on cytokine production by B cells also has been investigated. Epratuzumab inhibited the secretion of the pro-inflammatory cytokines TNF and IL-6 after anti-BCR or anti-BCR plus CpG stimulation of purified blood B cells from both healthy donors and SLE patients [119]. In contrast, the production of IL-10 by B cells (or induction of $IL\text{-}10^{+}$ cells) was not significantly affected by epratuzumab under any conditions, although the balance between pro-inflammatory and regulatory cytokines (IL-6/TNF versus IL-10) was altered in favour of the latter. In this regard, epratuzumab enhanced production of IL-10 by human tonsil naïve B cells stimulated with anti-BCR plus R848 (a TLR7 agonist on B cells) [63]. There was a donor-dependent relationship between the absolute expression of the TLR7 receptor and the capacity for B cells to produce IL-10, which may contribute to the heterogeneity of the response and the capacity for epratuzumab to affect that response.

8. Pharmacodynamic studies in SLE patients are providing insights into the functional impact of epratuzumab on B-cell function

As one might predict based on the in vitro data already discussed, an initial pharmacodynamic effect observed in patients treated with epratuzumab might be the loss of CD22 from the B-cell surface [100,120]. The maximal effect (approximately 70–80% loss relative to predose) was noted one week after the first dose of epratuzumab, was maintained throughout the treatment period, and was observed on naïve, memory/activated and transitional B-cell subsets. Furthermore, CD22 levels remained low for more than 2 years in patients receiving long-term treatment with epratuzumab [120]. However, the effect appeared to be reversible: a return to baseline value was observed after dosing ceased, at least at the lower doses, although it is not clear whether this represents the expression of new CD22 protein on circulating B cells or simply the release of new B cells into the periphery that had not internalised their CD22. It should be noted that approximately 20–30% of CD22 remains on the B-cell surface and cannot be further reduced by increasing doses of epratuzumab, suggesting that a subset of CD22 molecules exists that cannot be dynamically regulated by epratuzumab. Finally, in keeping with in vitro data, B cells from SLE patients treated with epratuzumab show a reduction in their expression of CD19, which may contribute to epratuzumab's activity in SLE patients [100].

A second pharmacodynamic observation noted in epratuzumab-treated patients was a reduction of B-cell numbers in the blood, although this effect is moderate and the kinetics protracted. In a small open-label study [30], where 12 SLE patients received 4 doses of epratuzumab, a maximum decline of approximately 30% of total B cells was noted, and $CD27^-$ naïve B cells appeared to be more affected that $CD27^+$ memory B cells. Another small study in patients with Sjögren's syndrome noted a mean reduction of around 50% after 4 infusions [121]. In a subsequent larger SLE study, very small changes were noted after a single cycle of treatment with epratuzumab, although there was evidence for a small decrease in the proportion of $CD22^+$ naïve B cells and a commensurate increase in the proportion of $CD22^+$ memory B cells [120]. Data from the SLE open-label extension study demonstrated that a median 50–60% reduction of total B cells occurred after 9–12 months and that this did not increase beyond that time point, even in patients receiving epratuzumab for up to 3 years.

Overall, these data suggest that, unlike rituximab, epratuzumab is not a B-cell-depleting antibody. Rituximab targets CD20 on B cells and is capable of causing an almost complete removal of at least blood B cells in a matter of days, which is often sustained for long periods of time [136], although the presence of recently generated IgA^+ plasmablasts indicates that this agent does not completely inhibit B-cell precursors [122]. In vitro studies demonstrate that epratuzumab is unable to induce complement-dependent cytotoxicity (CDC) or direct apoptosis of B cells in culture, and induces only modest antibody-dependent cellular cytotoxicity (ADCC) [114,123], in contrast to rituximab, which can mediate all three depletion mechanisms. This lack of effector-driven B-cell depletion is probably a consequence of the propensity of epratuzumab to induce internalisation or trogocytosis of CD22, making it an unsuitable target antigen to act as an 'anchor' for effective cell ablation [124]. In a similar manner, the capacity of different anti-CD20 monoclonal antibodies to induce cell depletion in vitro is inversely related to their capacity to be internalised [125].

With this in mind, it is pertinent to ask for an explanation for the B-cell reductions observed in patients receiving epratuzumab. It has been established that the normal maturation of B cells requires BCR signals both in the presence of antigen or its absence ('tonic' signalling) such that, for example, removal of the BCR or deletion of CD79α/β prevents maturation of B cells or causes them to revert to a less differentiated phenotype, or to be ablated [126–129]. Indeed, the expression of the BCR appears to be necessary for the survival of all B cells [69]. Given the potential induction of sustained, chronic inhibition of BCR signalling, it is possible that epratuzumab curtails the normal pathway of B-cell maturation or survival. Notably, mice with mutated non-functional ITIM residues on CD22 have enhanced maturation of transitional to mature B cells [130] and accelerated proliferation of naïve B cells following antigen stimulation, resulting in rapid generation of plasmablasts/plasma cells and faster antibody production [131]. Another hypothesis for reduced B cells relates to possible changes in B-cell migration and trafficking into different tissue compartments. For example, the VLA4/CXCL12/CXCR4 "zip code" serves to keep immature B cells in the bone marrow and prevents further maturation [132,133]. In this regard, it has been shown that epratuzumab enhances expression and function of β1 integrins (notably α4β1) on B cells and increases their migration to CXCL12 [62]; therefore, an epratuzumab-mediated altered migratory capacity could be operative in preventing release and/or maturation of immature B cells in the bone marrow. Furthermore, the organisation of germinal centres into dark and light zones appears to depend on opposing gradients of CXCL12 and CXCL13 [134], and it has been speculated that VLA4 ligands might be expressed at higher levels in the germinal centres [135], raising the intriguing possibility for modulation of B cells within the germinal centre.

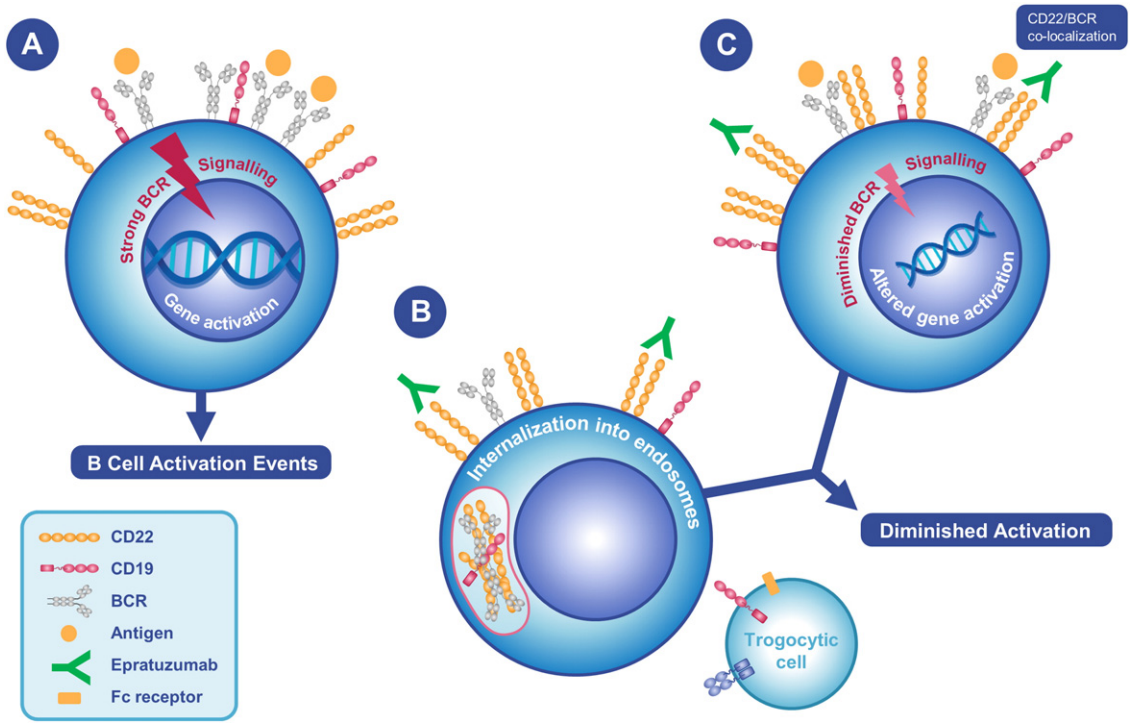

Fig. 1. The proposed unique mode of action of epratuzumab: BCR modulation leading to diminished B-cell activation. A: BCR engagement with antigen normally triggers a cascade of intracellular phosphorylation events (e.g., Syk, PLCγ2) and changes in Ca^{2+} flux that eventually activate downstream signals (e.g., NFκB, ERK) which then initiate changes in gene activation, ultimately driving B-cell activation; CD19, as a positive regulator of the BCR, further amplifies B-cell signalling capacity. B: In resting B cells, epratuzumab induces physical down-regulation of BCR-related proteins, such as CD19 and CD22 itself, from the B-cell surface through mechanisms such as internalisation and trogocytosis, leading to an altered BCR complex which would likely result in less responsive B cells. C: epratuzumab also induces direct co-localisation of phosphorylated CD22 (and SHP-1) with the BCR and limits the extent of BCR signalling after BCR engagement, resulting in diminished B-cell stimulation. The overall impact is inhibition of B-cell functional activation, including decreased proliferation and pro-inflammatory cytokine production, ultimately reducing subsequent autoimmune and inflammatory events mediated by B cells.

Regardless of the mechanism, whether epratuzumab influences a particular checkpoint in the maturation of B cells has yet to be fully defined. However, it is clear that understanding precisely how B cells are changed in patients in response to epratuzumab therapy is limited by the fact that we are effectively restricted to monitoring changes in the blood compartment, whilst the real 'site of action' is likely to be the lymphoid tissues or affected organs that are not readily available for study. As discussed earlier in this review, there is evidence for a number of B-cell abnormalities in the SLE blood compartment, and these actually are amenable to investigation. For example, the $CD27^-/IgD^-$ double-negative memory B-cell population that expresses high levels of CD95 is known to be elevated in SLE patients with disease flares and correlate with disease activity and serologic abnormalities [30]. Interestingly, there is a gradual decline in the numbers of these cells after long-term treatment with epratuzumab [120].

9. Conclusions

Activated B cells are believed to play a role in the pathogenesis of SLE by inducing several autoimmune/inflammatory processes, which include the production of autoantibodies, antigen presentation, support of T-cell differentiation, and/or production of pro-inflammatory cytokines. The key aspects of the mechanism of action of epratuzumab are summarised in Fig. 1, and indicate that this agent in essence enhances the normal inhibitory function of CD22 on the BCR. Firstly, by targeting CD22, epratuzumab induces physical down-regulation of BCR complex components on resting cells, which would likely result in less responsive B cells. Secondly, epratuzumab limits the extent of BCR signalling after BCR engagement. The overall effect is inhibition of B-cell activation, ultimately reducing subsequent autoimmune and inflammatory events mediated by B cells. It is thus intriguing to speculate how epratuzumab may also be therapeutically active in other B-cell-implicated autoimmune diseases.

Conflicts of interest

Thomas Dörner has received research grants and consultancy fees from UCB Pharma.

Anthony Shock is an employee of UCB Pharma.

David M. Goldenberg is an employee of Immunomedics Inc.

Peter E. Lipsky has received consulting fees from UCB Pharma.

Take-home messages

- The B cell remains an attractive target for new therapies to address the unmet clinical need in patients with systemic lupus erythematosus (SLE), an autoimmune disease associated with dysregulated immune function and hyperactivated B cells.
- CD22 plays a key role in modulating the functions of the B-cell receptor (BCR), a receptor complex on the B-cell surface that plays a critical role in B-cell development, activation and survival, thus regulating the ultimate fate of B cells.
- The monoclonal antibody epratuzumab targets CD22 and its mechanism of action in SLE involves perturbation of the BCR signalling complex and intensification of the normal inhibitory role of CD22 on the BCR, leading to reduced signalling and diminished activation of B cells.

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