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# Optimising B-cell depletion in Autoimmune Disease: Is Obinutuzumab the answer?

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**Abstract (100)**

In Rheumatoid Arthritis (RA) and Systemic Lupus Erythematosus (SLE), B-cell depletion therapy using rituximab results in variable clinical responses between individuals, which likely relates to variable B-cell depletion in the presence of immune defects. Outcomes in clinical trials with other type I anti-CD20 mAbs, ocrelizumab and ofatumumab, are comparable to rituximab. A mechanistically different type II mAb, obinutuzumab (OBZ), with greater capacity for B-cell depletion, has recently entered clinical trials in SLE. Here we consider whether type II anti-CD20 mAbs will provide mechanistic advantages to overcome the disease-related immune defects in autoimmune diseases such as SLE.

**25-30 word teaser**

Will the type II anti-CD20 mAb, obinutuzumab, provide mechanistic advantages over the type I mAbs, rituximab ocrelizumab and ofatumumab,

leading to improved B-cell depletion and clinical responses in autoimmune diseases?

## Introduction

B-cell depletion therapy with rituximab (RTX), a mouse-human chimeric anti-CD20 monoclonal antibody (mAb) improves outcomes for patients with B-cell malignancies and, for over a decade now, has also been used in the management of autoimmune diseases ranging from RA and SLE to ANCA-associated vasculitis and dermatomyositis (1, 2). A better understanding of the mechanisms of action and resistance of CD20 mAbs in the context of B-cell malignancies, has helped the development of more efficient anti-CD20 mAbs leading to improved clinical responses, as reviewed previously (3-6). However, differences between malignant and autoimmune B-cells including their number, size, location and role in pathogenesis as well as immune abnormalities associated with autoimmune disease mean that careful consideration is required if we are to make progress in optimising B-cell depletion for autoimmune disease.

## B-cell targeting in RA and SLE: trials and tribulations

B-cells play a critical role in the pathogenesis of chronic, multi-system, autoimmune diseases such as RA (7) and SLE (8). Therefore, B-cells are logical therapeutic targets in these diseases (1). Although selective targeting of only the pathogenic B-cells, leaving the immunoregulatory B cells intact, is desirable it remains unachievable given the lack of reliable markers that clearly distinguish pathogenic from non-pathogenic B-cells. B-cells may be targeted through deletion or through modulation of their function and/or survival. Inhibition of B-cell survival and/or function using belimumab, an anti-B-cell activating factor (BAFF) receptor mAb appears to be effective in SLE (9) and to a limited extent in RA (10) leading to the FDA approving its use for refractory SLE. Alternatively, modulation of B-cell function and modest B-cell depletion, using epratuzumab, an anti-CD22 mAb, does not appear to be effective in the management of SLE in recent phase III trials (11). By contrast, there is substantial evidence on the safety and efficacy of B-cell depletion therapy in refractory RA (12, 13).

Whereas clinical efficacy of RTX was demonstrated in randomised controlled trials (RCT) in refractory RA (12) and ANCA-associated vasculitis (14) two RCTs failed to demonstrate efficacy in SLE (9, 15). Although a number of factors including trial design may offer some explanations for this failure and apparent lack of efficacy of RTX in SLE (16), the frequently observed incomplete B-cell depletion in SLE (17-19) may well contribute to poor clinical response. Lack of effective depletion is also associated with poorer response in RA (17). Therefore, it is of clear clinical importance to better understand the mechanisms of action and resistance of anti-CD20 mAbs in the context of autoimmune disease to improve B-cell depletion in RA, and SLE in particular. Meanwhile, it will also be important to understand how the B cell depleted environment affects T cell responses in each case. Although clinically appropriate for a variety of rheumatic diseases including those that are antibody mediated, use of rituximab in autoantibody negative and T cell mediated autoimmune diseases such as psoriasis and seronegative spondyloarthropathies is uncertain. Recent findings show that RTX exhibited significant efficacy in psoriasis/psoriatic arthritis patients with long-standing disease(20, 21), but studies to confirm clinical efficacy are warranted. On the contrary, occurrence of psoriasis in patients receiving RTX for RA, SLE(22) and non Hodgkin lymphoma(23) has also been reported showing that removal of B cells may even precipitate T cell mediated autoimmunity in some cases.

Lessons learnt from studies in B-cell malignancies reveal that depletion with anti-CD20 mAbs is entirely dependent on the immune system of the host (4). Immune abnormalities are frequently associated with autoimmune disease and therefore could potentially impact on the effector mechanisms of anti-CD20 mAbs (Figure 1). The relevance of the various abnormalities will be discussed below along with whether the next generation of anti-CD20 mAbs will provide mechanistic advantages in the treatment of these autoimmune diseases.

**Lessons from non-malignant B-cell depletion with rituximab**

Several groups have shown that variability in RTX-induced B-cell depletion, at least in part, relates to the variability in clinical response in patients with RA and SLE. Early studies demonstrated that variability in B-cell depletion between individuals occurs regardless of the dose of RTX, in both RA and SLE (17, 24) and that poor clinical response is more common in those with incomplete depletion (19, 24) raising important questions about whether low serum RTX levels and/or the development of human anti-chimeric antibodies (HACAs) reduced its efficiency.

Is poor B-cell depletion simply due to low rituximab levels? The pharmacokinetics of RTX between malignancies and autoimmune disease are not directly comparable, not least due to differences in target cell number and size, but also due to differences in the dosing regimens. Our group has shown that, despite using the same dosing regimen, serum RTX levels are significantly lower in SLE compared to RA regardless of the presence of proteinuria or lupus nephritis (25), perhaps due to greater IgG catabolism contributing to reduced serum half-life of IgG in SLE compared to RA (26). Therefore, accelerated clearance of rituximab remains potentially an important resistance mechanism in SLE.

In relation to this, is poor B-cell depletion due to the development of human anti-chimeric antibodies (HACAs)? HACAs may antagonise rituximab and increase clearance in vivo (27) and a higher frequency of HACAs are reported in SLE, with up to 26% of 169 patients enrolled in a randomised controlled trial developing HACAs over a 52 week period (15) compared to 4.3-11% of patients with RA (12, 28, 29). Regardless, HACAs do not appear to determine clinical response in RA (12, 29) or SLE (15). Furthermore, humanized anti-CD20 mAbs do not appear to deliver better clinical outcomes than rituximab in autoimmune disease, although there is no directly comparable data (Table 1). Therefore, alternative resistance mechanisms need consideration in our efforts to improve B-cell depletion in autoimmune diseases.

There seems to be some disparity in the extent of B-cell depletion according to anatomical location such that a complete depletion of non-malignant B-cells was noted more frequently in peripheral blood compared to tissues such as the bone marrow (30), lymph nodes (31, 32) and synovium (33, 34). Thus location seems to influence depletion perhaps due to circulatory dynamics contributing to impaired access for effector mechanisms (35). In part, this could also relate to differing B-cell subpopulations in these locations, which may vary in their susceptibility to depletion with RTX? Whereas IgD+CD27- naïve B-cells are completely depleted by RTX in peripheral blood in both RA and SLE, low numbers of IgD-CD27+ switched memory B-cells are detectable in some patients, particularly in those with poor clinical response (36, 37). Also, in RA, a greater frequency of switched memory (IgD-CD27+) B-cells is noted in the synovium compared to peripheral blood after RTX (34). Thus, there seems to be a disparity in the susceptibility of different non-malignant B-cell subpopulations to depletion with rituximab. Furthermore, patients who were treated with low dose (500mg) RTX, as part of pre-conditioning before kidney transplant to reduce graft rejection, demonstrated complete B-cell depletion in peripheral blood, but had a greater frequency of switched memory (IgD-CD27+) B-cells in lymph nodes compared to those not treated with RTX, whereas the frequency of naïve (IgD+CD27-) B-cells and unswitched memory (IgD+CD27+) B-cells was lower (31, 32) suggesting that both location and B-cell intrinsic mechanisms confer resistance to depletion with RTX. Interestingly, failure to deplete in lymph nodes is not due to lack of opsonisation as non-deleted B-cells have surface bound RTX and in vitro they remain functional even in the presence of low concentrations of RTX (38) (31). Thus, IgD-CD27+ switched memory B-cells appear to be more resistant to depletion with RTX.

There is however no data yet on the extent of B-cell depletion in secondary lymphoid tissues in relation to clinical response to RTX in patients with RA and SLE. Thus, while achieving complete B-cell depletion in RA and SLE appears to be clinically relevant, how some B-cells evade deletion by RTX, particularly in lymph nodes, remains elusive. Studies in B-cell malignancies

identified several potential resistance mechanisms that contribute to ineffective B-cell depletion with RTX, reviewed previously (3-5, 35, 39). Here, we will focus on the mechanisms of action of anti-CD20 mAbs and discuss how immune abnormalities associated with autoimmune disease may potentially impact on these.

## Anti-CD20 mAbs: effector mechanisms

Anti-CD20 mAbs evoke four distinct effector mechanisms to delete target B-cells: antibody-dependent cellular cytotoxicity (ADCC); antibody-dependent cellular phagocytosis (ADCP); complement-dependent cellular cytotoxicity (CDC); and direct cell death (DCD). Evidence for the mechanisms of B-cell depletion by RTX in RA and SLE is limited and therefore we will focus on studies in B-cell malignancies and mouse models and use these to infer how disease-associated defects could impact on the efficiency of CD20 mAbs in autoimmune conditions.

A key characteristic that distinguishes CD20 mAbs is the ability to cluster CD20 into detergent insoluble lipid rafts. Type I CD20 mAbs like RTX, are significantly more efficient at redistributing CD20 into lipid rafts compared to type II CD20 mAbs like obinutuzumab (39-42). The importance of this lies in the observation that redistribution of CD20 positively correlates with the ability of CD20 mAbs to evoke CDC (40) while increasing the propensity for internalization of CD20:CD20 mAb complexes by B-cells (42). Whereas efficient activation of CDC is desirable for B-cell deletion, internalization of CD20:CD20 mAb complexes is detrimental to the other effector mechanisms for B-cell deletion (39, 41, 43). A number of type I CD20 mAbs have been used in clinical trials in RA (44) and/or SLE (Table 1) but OBZ is the only type II mAb to have entered clinical trials in SLE. The key question is whether it matters what type of anti-CD20 mAb is used in autoimmune disease and whether type II will improve responses.

## Could immune abnormalities associated with autoimmune diseases impair the efficiency of CD20 mAbs?

*Complement-dependent Cellular Cytotoxicity*

Complement is rapidly consumed in patients with chronic lymphocytic leukaemia (CLL) after RTX infusion inferring complement activation in-vivo (45). Replacement of complement using fresh frozen plasma enhances the efficiency of RTX in in vitro complement assays using human CLL samples (46). It was also shown that RTX activity is dependent on complement in some animal models (47). However, complement does not appear to be important for B-cell depletion in FL, where there is no correlation between B-cell depletion and the expression of complement defence molecules CD45, CD55 and CD59 (48) and other studies also suggest that there is little requirement for complement for RTX-induced B-cell depletion in vivo (41, 49). It has been suggested that CDC depends on target cell expression of CD20 (50), which may be overcome by cytokine treatment to induce higher CD20 expression on B-cells (51).

Complement has also been considered detrimental to B-cell depletion in some cases as deposition of C3b on CD20:RTX complexes may promote their ‘trogocytosis’ by immune cells (52, 53), and may also block the interaction between the Fc portion of RTX and CD16A (FcγRIIIA) on NK cells (54), potentially inhibiting ADCC. Consistent with the concept of complement hindering the efficiency of RTX, it was shown that FL patients with a C1qA polymorphism associated with low C1q levels displayed greater clinical responses with RTX compared to those with high C1q levels associated with the alternate allele (55). Thus, current literature does not support a prominent role for CDC in determining RTX efficacy.

Complement defects and C1q deficiency in particular is characteristic of SLE (56) (57). Could SLE-associated defects in complement impact on the efficiency of RTX? In this regard it was shown that in murine models with C1q deficiency, B-cell depletion with RTX was not significantly effected (41). Also, there is no direct evidence to support that B-cell depletion is less efficient in patients with SLE and those with low complement levels and high anti-double-stranded DNA antibody levels respond well to RTX therapy (15). Regardless, if CDC is a key effector mechanism for non-malignant B-cell depletion in vivo, the efficiency of type I mAbs like RTX, but not type II mAbs like OBZ, would be compromised by SLE-associated

complement defects. Therefore in this regard OBZ may provide a mechanistic advantage in SLE.

*Fc:Fc$\gamma$R dependent Effector Mechanisms*

The critical importance of Fc:FcγR dependent effector mechanisms, ADCC and ADCP, for in vivo B-cell depletion was highlighted in animal models using malignant B-cell xenografts (49). ADCC and ADCP are both dependent on the interaction between the Fc portion of mAb and FcγR on effector cells including NK cells, neutrophils, monocytes and macrophages. Current knowledge of which effector cell is key for B-cell depletion in vivo is mainly derived from studies in animal models and strengthened by indirect evidence of associations between genetic polymorphisms in patients with B-cell malignancies and autoimmune diseases and the clinical response to RTX. Current evidence favours that myeloid cells and likely macrophages are the key effectors, at least in mice (58). So, what other factors affect anti-CD20 mAb-induced ADCC/ADCP?

In vitro, antigenic modulation of CD20 from the surface of normal and malignant B-cells appears to, at least partly, account for the inferior efficiency of RTX at inducing Fc:FcγR dependent effector mechanisms compared to type II anti-CD20 mAbs like OBZ (39) (51). We have previously shown that the inferior efficiency of RTX compared to OBZ-WT, a type II mAb with glycosylated Fc, as assessed in whole blood B-cell depletion assays using samples from patients with RA and SLE is, at least partly, attributable to internalization of RTX (59). Thus, CD20 modulation by mAbs is a key resistance mechanism specific to type I mAbs like RTX and ofatumumab.

*Antibody-dependent cellular cytotoxicity*

What factors associated with RA and SLE regulate ADCC? NK cells are key mediators of ADCC and defects in NK cell function are described both in RA and SLE (60, 61). Cytokine abnormalities associated with SLE such as increased levels IFN$\alpha$ and IFN$\gamma$ (62) appear to inhibit the cytotoxic

function of NK cells, and consequently, ADCC (63, 64). Intriguingly, interferon signature is described as a marker of poor response to RTX in patients with RA (65-67), although alternative explanations including effects of IFNγ on the generation of plasmablasts require consideration (68). Also corticosteroids, commonly used in the management of both RA and SLE may also inhibit optimal ADCC by inhibiting the release of cytotoxic granules such as perforin and granzyme from NK cells (69). Thus, both disease-associated elevated levels of cytokines and corticosteroid therapies used for the management of RA and SLE may independently inhibit the cytotoxic function of NK cells, consequently, compromising the efficiency of RTX.

The 158V genetic polymorphism in FcγRIIIa, resulting in higher affinity binding to IgG1 is associated with better clinical response to RTX in FL (70), but not CLL (71), suggesting that at least in some diseases Fc:FcγR-dependent effector mechanisms are key for the efficacy of RTX. In both RA and SLE the FcγRIIIa 158V polymorphism is associated with better clinical response to RTX (72, 73) providing indirect evidence for the importance of Fc:FcγR-dependent effector mechanisms in these conditions. In ADCC assays using effector cells from healthy individuals homozygous for the lower affinity 158F FcγRIIIa polymorphism it was shown that the efficiency of RTX was inferior to OBZ (74). Therefore, in this context, OBZ with an afucosylated Fc portion may overcome the limitation of the low affinity FcγRIIIa 158F allele in some individuals with SLE and prove advantageous.

*Antibody-dependent cell phagocytosis*

ADCP is considered a critical effector mechanism evoked by anti-CD20 mAbs with neutrophils, küpffer cells and macrophages serving as effectors. In mice, küpffer cells in the liver play an important role in the deletion of B-cells following anti-CD20 mAb (75). However, in humans, the data on the role of küpffer cells in the liver participating in ADCP is currently limited, and so will not be discussed further here.

With regards to other phagocytes, neutrophils are the major leukocytes in peripheral blood and whole blood assays have revealed that they can mediate phagocytosis of mAb opsonised B-cells (76, 77). Given the high level of homology between FcγRIIIa and FcγRIIIb, Fc modification through afucosylation also result in higher affinity binding to FcγRIIIb on neutrophils. As a result, glycoengineered RTX and OBZ, are shown to induce greater neutrophil-mediated phagocytosis of mAb opsonised B-cells in whole blood assays compared to non-glycoengineered RTX (77). Although polymorphisms of FcγRIIIb do not correlate with clinical response in FL (78), concurrent administration of GMCSF with RTX improves clinical response (79). However, improvements in clinical response with GMCSF are probably not limited to effects on neutrophils but also attributable to other effector cells such as monocytes and macrophages. Therefore, to what extent neutrophil-mediated phagocytosis determines mAb efficacy is not clear. Furthermore, in some lupus patients, clearance of neutrophil extracellular traps (NETs) is inefficient(80). Formation of NETs is regulated by FcγRIIIb cross linking(81) and provides a potent and continuous stimulus for type 1 interferon release contributing to lupus pathogenesis. It will therefore be important to assess how neutrophil ‘NETing’ is influenced by RTX and type II antibodies.

Although different FcγR expressing effector cells are probably involved in mAb mediated ADCP, macrophages appear to be the key effectors. Evidence from animal studies using targeted deletion of effector cells, suggests that the main effector cells for in vivo B-cell depletion are macrophages, not NK cells or neutrophils (35, 43, 47, 49, 82, 83). In humans, high numbers of tumour-associated macrophages is predictive of poor prognosis in patients treated with chemotherapy alone, but not in patients treated with RTX (84) suggesting that RTX treatment is facilitated by these macrophages, partly overcoming the chemotherapy-resistance effects. Also the correlation between FcγRIIa 131H polymorphism with clinical response to RTX in FL (85) provides indirect evidence for the role of macrophages as key effectors because the majority of macrophages and

very few (if any) NK cells express FcγRIIa (86). These findings suggest that macrophages are probably the key effector cells that mediate RTX-induced B-cell depletion in B-cell malignancies.

In vitro assays suggest that high levels of immunoglobulin, such as typically described in SLE, may impair the efficacy of RTX to a greater extent compared to OBZ, presumably by inhibiting Fc:FcγR dependent effector mechanisms (87) and this is supported by animal studies in a lupus-prone mouse model (88). As yet there is no evidence on whether, if at all, the level of hypergammaglobulinemia described in SLE adversely affects the efficiency of anti-CD20 mAbs. However, as afucosylation increases the affinity of IgG for FcγRIIIa on effector cells, it may overcome competition from the high levels of immunoglobulin resulting from hypergammaglobulinemia and perhaps yield mAb which perform better at lower concentrations in vivo, as shown in vitro (39, 77, 87). Alternatively, engineering of mAbs with amino acid substitutions in the Fc portion (e.g. G236A) designed specifically to increase IgG1 affinity in favour of FcγRIIa relative to FcγRIIb may promote phagocytosis of mAb-opsonised B-cells by macrophages (89). However, whether such Fc engineered mAbs will increase the efficiency of mAb-mediated Fc:FcγR dependent effector mechanisms and improve clinical response remains to be proven.

*Direct Cell Death*

Currently, our understanding of the importance of DCD elicited by anti-CD20 mAbs is primarily derived from studies in malignant cell-lines because evidence of DCD as an effector mechanism in vivo in humans is limited. The pathways to DCD by mAbs include induction of caspase-dependent apoptotic cell death upon hyper-crosslinking of RTX (90), and lysosome-mediated cell death in the case of type II mAbs like OBZ, which is activated in the absence of further crosslinking (41) (91). In vivo, FcγR-bearing effector cells are proposed to deliver crosslinking of mAbs bound to B-cells (5) but whether this delivers DCD is unclear. In support, there is some evidence of caspase activation in samples from patients with CLL

treated with RTX suggesting induction of apoptosis in vivo (92). Expression of anti-apoptotic proteins such as MCL-1 is reported in other B-cell malignancies and may be associated with resistance to RTX (93, 94).

Non-malignant B-cell subpopulations differ in their expression of anti-apoptotic proteins such as Bcl-2 such that switched (IgD-CD27+) memory B-cells express relatively higher levels than naïve (IgD+CD27-) B-cells (95, 96). Therefore, switched (IgD-CD27+) memory B-cells may offer greater resistance to RTX-mediated apoptotic DCD. Further, in SLE, the levels of B-cell activating factor (BAFF), are high and BAFF-mediated anti-apoptotic effects (97) may also offer another layer of additional resistance to RTX whereas type II mAbs like OBZ induce lysosome-mediated DCD (independent of intrinsic apoptosis) and therefore the ability of OBZ to induce DCD in non-malignant B-cells is less likely to be hindered by the expression of anti-apoptotic proteins.

Opsonisation with RTX does not always induce B-cell depletion in vivo (31, 32). This raises the question as to what extent DCD serves as an effector mechanism for RTX in vivo and whether OBZ may induce greater DCD as shown to be the case in vitro (74). In vitro, RTX induces modest levels of DCD in both IgD+CD27- naïve and IgD-CD27+ memory B-cell subpopulations in samples from patients with RA (90). Regardless, DCD may be an important effector mechanism particularly when other effector mechanisms such as CDC, ADCC and ADCP are compromised due to deficiencies in complement, lack of access to target cells or inherent or acquired defects in effector cells.

Taken together, a number of SLE-associated immune abnormalities could potentially impair all four effector mechanisms evoked by anti-CD20 mAbs, as shown in Figure 1.

**Other resistance mechanisms**

Similar to malignant B-cells, non-malignant B-cells may potentially evade cytotoxicity by anti-CD20 mAbs by a number of mechanisms including low

level of expression of target antigen CD20, internalisation of CD20:mAb complexes and/or by removal of CD20:mAb complexes by effector cells through trogocytosis. Whilst a significant variability in the expression of CD20 between B-cells in different compartments or among different B-cell subpopulations may influence their susceptibility to removal by RTX, as yet no such data is available. However, as demonstrated in malignant B-cells (42), we have shown that, in vitro, B-cells from patients with RA and SLE internalize RTX impairing its efficiency in whole blood B-cell depletion assays (59). Alternatively, CD20:RTX complexes may also be 'shaved' from the cell surface through trogocytosis as initially demonstrated for malignant B-cells by the Taylor laboratory (52) and also shown in samples from patients with RA (98). However, to what extent trogocytosis of CD20:RTX complexes reduces mAb efficiency in vivo remains to be conclusively proven. Thus, resistance mechanisms identified in the context of B-cell malignancies may also operate in autoimmune disease.

**What next for optimising B-cell depletion strategies in autoimmune diseases?**

The available data clearly demonstrate that RTX-based B-cell depletion therapy improves outcomes for the majority of patients with autoimmune diseases such as RA and SLE. However, a significant proportion of patients that do not respond optimally likely due to the various resistance mechanisms outlined above. Although alternative, humanised, anti-CD20 mAbs have now entered clinical trials for use in RA and SLE, some demonstrating efficacy in RTX-naïve RA, it is not yet clear whether they will be more effective as currently there is no data comparing these mAbs in head-to-head trials with RTX. It is also currently not known whether they will also be effective in RTX-refractory patients. Presumably this will depend upon the reason for resistance in the first place. If development of HACA or anti-idiotype responses is responsible, then alternative mAb should be effective. Alternatively, if a patient-intrinsic mechanistic defect is responsible, then unless this is overcome with the new treatment no improvement in response would be expected. In interpreting the head-to-head trials it is important to note that the doses used may not be directly

comparable to those for RTX in routine clinical practice (either two doses of 1g given 1-2 weeks apart or four doses of 375-mg/$m^2$ given weekly). This point is particularly pertinent given that RTX resistance may, at least in part, be overcome by using an extra dose of RTX as shown in the case of RA (18). Another important factor needing careful consideration is to distinguish the biological activity of these anti-CD20 mAbs from their clinical activity. For example, in some patients efficient depletion may not always lead to good clinical response, conversely, good clinical response may occur despite inefficient B-cell depletion. For this reason it will be important to monitor these activities separately and where possible perform mechanistic studies involving patients in clinical trial settings to provide clinically meaningful information and provide insights into how best to optimise B-cell depletion therapy further. Given its enhanced ability to delete B-cells in other diseases and capacity to elicit alternative effector functions, we remain optimistic that the type II anti-CD20 mAb obinutuzumab may provide a potential solution in at least some patients.

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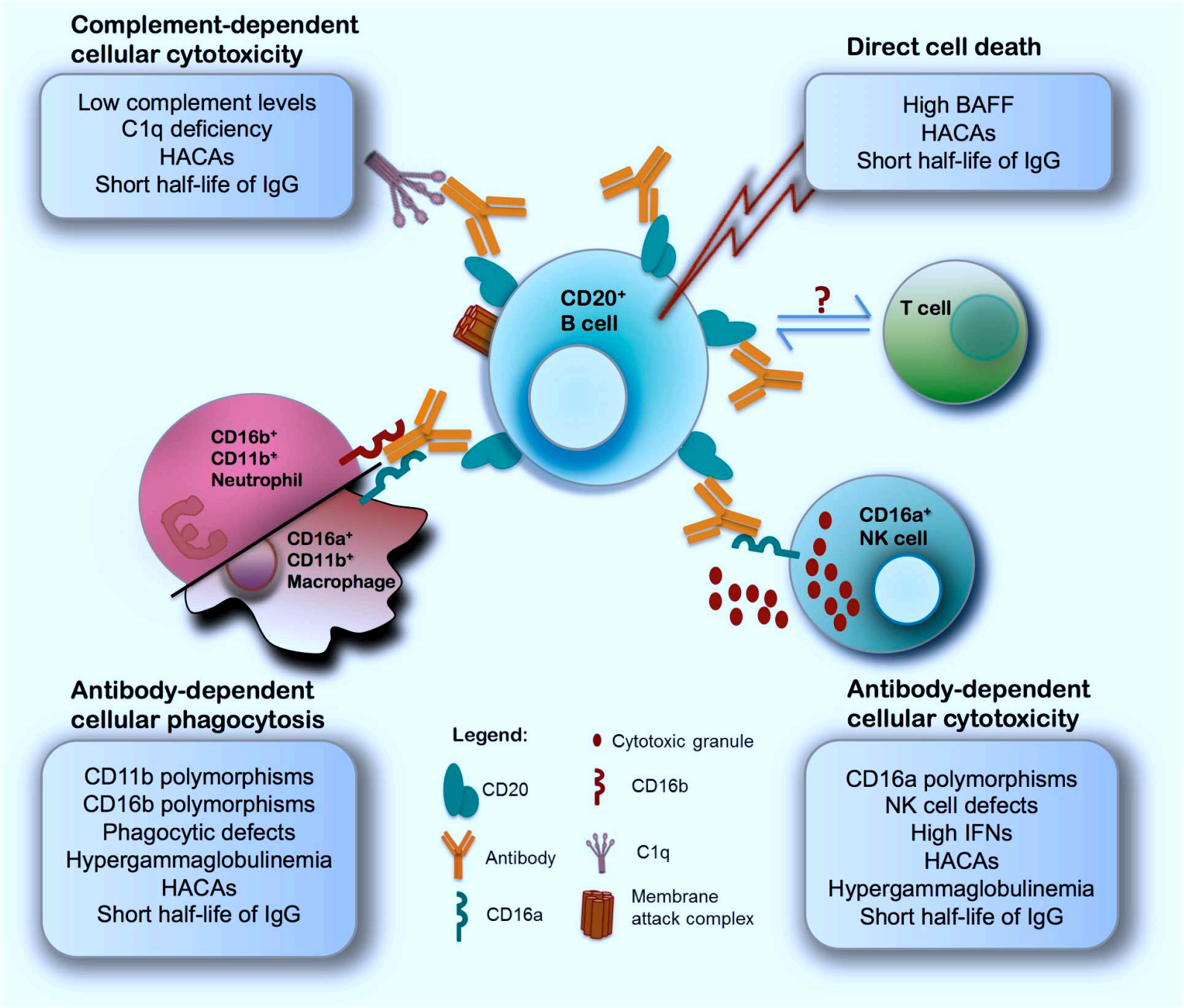
## Figure Legends

**Figure 1. Effector mechanisms of anti-CD20 mAbs and how SLE-associated immune abnormalities may influence them.** In each corner, the figure illustrates the four key effector mechanisms engaged by anti-CD20 mAb and in the blue boxes below, how SLE-mediated immune abnormalities may impact them. Top left) Complement-dependent cellular cytotoxicity. Type I anti-CD20 mAb bind to B-cells, promoting clustering of target antigen and distribution of the associated mAb Fc domains to bind and activate C1q, stimulating the complement cascade, resulting in the potential deletion of the target cell via insertion of the membrane attack complex (MAC) into the target cell. Top right) Direct cell death. Membrane bound anti-CD20 mAb may trigger apoptosis by activation of caspases, as in the case of Type I anti-CD20 mAb or by eliciting a non-apoptotic lysosomal mediated cells death as in the case of Type II mAb. Bottom right) Antibody-dependent cellular cytotoxicity. ADCC is triggered when CD16a on NK cells interacts with the Fc portion of anti-CD20 mAb inducing the release of cytotoxic granules containing perforin and granzymes. Bottom left) Antibody-dependent cell phagocytosis. ADCP is triggered when activating Fc$\gamma$R (e.g. CD32a or CD16a on macrophages; CD16b on neutrophils) on phagocytic cells engage with anti-CD20 mAb opsonised to target B-cells. In each case the SLE-associated immune abnormalities that potentially affect the effector mechanisms are listed in the boxes below. Within this complex system the impact of the immune environment and B-cell depletion on the T cell compartment is unclear; but remains an area of much interest. As one example, a vaccine-effect may also operate as an effector mechanism when T cells become sensitised to recognise B cell antigens following anti-CD20 mAb therapy.
HACAs, human anti-chimeric antibodies; IFN, interferons; BAFF, B lymphocyte activation factor.

Figure


Complement-dependent cellular cytotoxicity
Low complement levels
C1q deficiency
HACAs
Short half-life of IgG
Direct cell death
High BAFF
HACAs
Short half-life of IgG
CD20+ B cell
?
T cell
CD16b+ CD11b+ Neutrophil
CD16a+ CD11b+ Macrophage
CD16a+ NK cell
Antibody-dependent cellular phagocytosis
CD11b polymorphisms
CD16b polymorphisms
Phagocytic defects
Hypergammaglobulinemia
HACAs
Short half-life of IgG
Legend:
CD20
Antibody
CD16a
Cytotoxic granule
CD16b
C1q
Membrane attack complex
Antibody-dependent cellular cytotoxicity
CD16a polymorphisms
NK cell defects
High IFNs
HACAs
Hypergammaglobulinemia
Short half-life of IgG

**Table**

Table1. Other anti-CD20 mAbs in clinical trials or in clinical use in RA and SLE

| CD20 mAb | Type | Disease | Sponsor | Stage of trial | Results | Reference |
|---|---|---|---|---|---|---|
| Ocrelizumab | I | RA | Genentech/ Roche/Biogen | Phase III | Primary end points met (48 weeks, dose-dependent serious infections) | (44) |
| Ofatumumab | I | RA | Genmab AC | Phase III | Primary end points met (24 weeks, no serious adverse events) | (99) |
| TRU-015 | I* | RA | Trubion/Pfizer | Phase I & II | Primary end points not met | (100, 101) |
| Ocrelizumab | I | SLE (LN) | Genentech/ Roche/Biogen | Phase III | Not superior to placebo | (102) |
| Obinutuzumab (GA101) | II | SLE | Glycart/Roche | Phase I/II | Commenced Nov 2015 | |
| Ocaratuzuamab (AME-133v, LY2469298) | I | RA | Mentrik Biotech/ Eli Lilly | Phase I | N/A | |

* TRU-015 is a single-chain Fv generated from 2H7 linked to human IgG1 but devoid of CH1 and CL domains. The original mAb was Type I (40); RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; LN, lupus nephritis; and N/A, not available.

**Table**

Table 2. Characteristics of type I and II anti-CD20 monoclonal antibodies

| | Type I | Type II |
|---|---|---|
| Established mAb | Rituximab (103)<br>Ofatumumab (104)<br>Ocrelizumab (105)<br>AME-133v46 (106)<br>PRO131921 (107)<br>TRU-015 (100, 101)<br>Velutuzumab (108) | Obinutuzumab (6)<br>Tositumomab (109)<br>11B8 (110) |
| In clinical or trial use in RA and/or SLE | Rituximab<br>Ofatumumab<br>Ocrelizumab | Obinutuzumab |
| Redistributes CD20 | Yes | No |
| Internalisation of anti-CD20-mAb complexes | Yes, but highly variable | To a small extent and significantly less than rituximab |
| Homotypic aggregation | Weak | Strong |
| CD20 tetramer to mAb binding ratio | 1:1 | 2:1 |
| CDC | potent | weak |
| ADCC | Yes | Yes |
| ADCP | Yes | Yes |
| DCD | Apoptosis | Non-apoptotic<br>lysosome mediated cell death |

* TRU-015 is a single-chain Fv generated from 2H7 linked to human IgG1 but devoid of CH1 and CL domains The original mAb was Type I(40). RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; CDC, complement-dependent cellular cytotoxicity; ADCC, antibody-dependent cellular cytotoxicity; ADCP, antibody-dependent cell phagocytosis; and DCD, direct cell death.

**Table**

Table 3. Disease-related immune abnormalities and potential effect on the efficiency of CD20 mAb effector functions

| Immune abnormality associated with disease | Effect on condition | Predicted effect on effector mechanism | Reference |
|---|---|---|---|
| C1q deficiency | Risk of SLE | CDC ↓ | (57) |
| FcγRIIb T232 | Risk of SLE | Reduced activity of FcγRIIB ADCP↑ | (111, 112) |
| FcγRIIb promoter haplotype 2B.1-386G-120T | Risk of SLE | Associated with increased expression of FcγRIIb on both lymphocytes and myeloid cells ADCC ↓ ADCP ↓ | (113) |
| FcγRIIIa 158F* (low affinity allele) | Risk of SLE | ADCC ↓ ADCP ↓ | (114-116) |
| FcγRIIIb polymorphisms and copy number variations associated with reduced expression/binding | Risk of SLE | Neutrophil ADCP ↓ | (117, 118) |
| rs1143679 variant of ITGAM gene encoding the R77H variant of CD11b | Risk of SLE | Reduced phagocytosis of iC3b opsonised targets by neutrophils, monocytes and macrophages ADCP ↓ | (119) (120) |
| nonsynonymous SNPs rs1143678, and rs1143683 of ITGAM encoding CD11b | Risk of SLE | Reduced phagocytosis of iC3b opsonised targets by neutrophils ADCP ↓ | (120) |
| CD11b ↑ and CD62L ↓ on neutrophils | Risk of vasculitis associated with RA | ADCP ↑ | (121) |
| Hypergammaglobulinaemia | Associated with SLE | ADCC / ADCP ↓ | (87) |
| IFN□ signature | Risk of SLE Poor response to | ADCC ↓ | (62, 65, 66) |

| | | | |
|---|---|---|---|
| | RTX in RA | | |
| Reticuloendothelial Fc-Receptor-mediated clearance defects | Defect correlates with disease severity in SLE | ADCP ↓ | (122) |

SLE, systemic lupus erythematosus; CDC, complement-dependent cellular cytotoxicity; ADCC, antibody-dependent cellular cytotoxicity; ADCP, antibody-dependent cell phagocytosis; SNP, single nucleotide polymorphism. * In some nomenclature the 158 residue in FcγRIIIa is defined as 176.

## Highlights

- Incomplete B-cell depletion in RA and SLE contributes to poor response with rituximab
- CD20 modulation by mAbs is a key resistance mechanism of type I mAbs like rituximab
- Obinutuzumab is not dependent on complement for efficient B-cell depletion
- Obinutuzumab may overcome limitations of low affinity variants of CD16a and CD16b
- SLE-associated immune abnormalities potentially impair CD20 mAb-effector mechanisms