REVIEW

# A new approach to comparing anti-CD20 antibodies: importance of the lipid rafts in their lytic efficiency

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**Abstract:** The view that B lymphocytes are pathogenic in diverse pathological settings is supported by the efficacy of B-cell-ablative therapy in lymphoproliferative disorders, autoimmune diseases and graft rejection. Anti-B-cell antibodies (Abs) directed against CD20 have therefore been generated, and of these, rituximab was the first anti-CD20 monoclonal Ab (mAb) to be applied. Rituximab-mediated apoptosis, complement-dependent cytotoxicity and Ab-dependent cellular cytotoxicity differ from one disease to another, and, for the same disease, from one patient to another. This knowledge has prompted the development of new anti-CD20 mAbs in the hope of improving B-cell depletion. The inclusion of CD20/anti-CD20 complexes in large lipid rafts (LRs) enhances the results of some, but not all, anti-CD20 mAbs, and it may be possible to include smaller LRs. Lipid contents of membrane may be abnormal in malignant B-cells, and could explain resistance to treatment. The function of these mAbs and the importance of LRs warrant further investigation. A detailed understanding of them will increase results for B-cell depletion in lymphoproliferative diseases.

**Keywords:** anti-CD20 antibodies, lymphocyte B, lipid rafts, B-cell disorders

## Introduction

B lymphocytes perform a variety of functions in the immune system. For example, they play a part in eradicating bacteria or viruses, and promote all antitumoral responses.[1] They may, however, run the risk of upregulating immune reactions. In autoimmunity, there is sufficient evidence to justify investigating B lymphocytes not only as antibody (Ab) producing cells at the end of the pathological sequence, but also in the early stages of pathophysiology.[2] These negative effects of B lymphocytes may be seen in graft rejection, which is associated with the production of Abs,[3] and in graft versus host (GVH) disease following allogeneic hematopoietic stem cell transplantation.[4] Moreover, there is anarchic proliferation of B lymphocytes in lymphoproliferative syndromes such as lymphomas[5] and chronic lymphocytic leukemia (CLL).[6] Not surprisingly, B-cell-ablative therapies have been proposed for B-cell non-Hodgkin's lymphoma (NHL), representing a major advance in its treatment. One of these therapies involves anti-CD20 monoclonal Abs (mAbs), which are directed against the CD20-expressing members of the B-cell lineage, from late pre-B-cells through mature B-cells. Rituximab (RTX) was the first mouse–human chimeric mAb to be approved by the Food and Drug Administration (FDA) for the treatment of relapsed, or refractory, low-grade NHL.[7] RTX is now used in other malignant proliferations,[8] and is approved by the FDA for the treatment of autoimmune diseases (AIDS)[9] and the prevention of graft rejection.[10] To summarize the current situation, over 500,000 patients (including children) have

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been treated with RTX, and the improved patient-reported outcomes and cost-effectiveness have led to the production of other anti-B-cell agents. As a consequence, humanized anti-CD20 Abs are currently being developed.[11–14]

The effectiveness of anti-CD20 mAbs for a given disease is variable, as reported in patients with NHL or CLL.[15–18] Furthermore, their self-efficacy differs from one B-cell disease to another. In fact, compared to observations with lymphoma, RTX has been shown to be weak when used as a single agent in CLL.[19,20] These data therefore suggest that the results could be affected by factors in B lymphocytes themselves. The present review endeavors to chronicle therapeutic indications of anti-CD20 mAbs in these malignancies, to analyze the mechanisms of their action and to distinguish extrinsic or intrinsic factors, particularly lipid rafts (LRs) that could possibly be modulating their activities.

# Diseases treated with rituximab
## Malignant B-cell diseases
### Non-Hodgkin B-cell lymphoma
Many clinical trials have been conducted as induction therapy for patients recently diagnosed with follicular lymphoma or who have suffered a relapse when RTX was used on its own, in combination with chemotherapy, or with other types of Abs. Generally speaking, these mAbs, or the related combination of drugs, prolong survival compared to chemotherapy alone.[21] Other trials have tested the efficacy of RTX in Waldenström's macroglobulinemia (WM) and multiple myeloma.[22] In fact, Demopoulos et al have claimed that combining RTX with other drugs is the most commonly used primary treatment for WM.[23] Diffuse large B-cell lymphoma (DLBL), which accounts for approximately 40% of NHLs in adults, is a type of aggressive lymphoma with a diffuse growth pattern and a high-intermediate proliferation index. Combining RTX with chemotherapy represents a breakthrough in the treatment of malignancies, and could become the new standard therapy in elderly and young low-risk patients.[24] In addition, treatment of Burkitt's lymphoma, marginal lymphoma and Hodgkin lymphoma with RTX is currently being investigated (Table 1).

Its success notwithstanding, around 50% of NHL patients resisted treatment with RTX in that they did not respond to it, or they suffered an early relapse of the original disease.

## Leukemias
These disorders are, in fact, bone marrow (BM) diseases. As in previous papers, we show the results of treating CLL and acute lymphocytic leukemia (ALL) with RTX. Many clinical trials have been conducted on CLL,[25] but the efficacy of RTX was weak compared to its effects with lymphoma. One explanation is that RTX reduces the membrane level of CD20 on CLL B-cells. This is reflected in the fact that fractionation of RTX into several low doses perpetuates the expression of CD20, and thereby prolongs the activity of the mAb.[26,27] Finally, the results of combining RTX with chemotherapy in the treatment of ALL appear to be encouraging, with acceptable levels of toxicity (Table 1).[28]

# Autoimmune diseases
## Autoimmune diseases where RTX is known to be efficient
A pilot study, RTX in AID, was set up in 2000 for the treatment of immune thrombocytopenic purpura. Objective clinical responses were observed in 30% of cases[29] and the beneficial effect of RTX was confirmed in others.[30,31] Furthermore, the use of RTX with methotrexate has been approved by the FDA for the treatment of rheumatoid arthritis (RA), as it relieves symptoms in adult patients with moderate to severe active RA where treatment with one or more anti-TNF drugs has failed. RTX was also tested in Sjögren's syndrome with interesting results,[32] and in systemic lupus erythematosus (SLE) where reduced activity of the disease and a clinical response of 86% were reported in one series of patients.[33,34] However, certain SLE

**Table 1** Diseases treated by rituximab

| | Auto-immune diseases (AID) | | | | | | | Stem cell or organ transplantation | | | | Non Hogkin Lymphomas | | | | HL | Leukemia | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ITP | RA | SS | SLE | MS | ANCA | DM/PM | Auto-graft | Allo-graft | GVHD | Renal rejection | DLBL | MALT | FL | MCL | | ALL | CLL | HCL |
| Clinical phase | II III | IV | II III | III IV | II | II III | II | II | Pilotal trial | I/II | II | III | III | III | I | II | II | II | II |

**AID:** ITP, Idiopathic thrombocytopenic purpura; RA, Rheumatiod, Arthritis; SS, Sjogren's syndrome; SLE, Systemic lupusErythematosus; MS, Multiple sclerosis; ANCA, Antineutrophil cytoplasmic antibodies; DM, dermatomyositis; PM, polymyositis; GVHD, graft versus-host disease.
**Lymphomas:** DLBL, diffuse large B cell lymphoma; MALT, Mucosa-associated lymphoid tissue lymphoma; MCL, Mantle cell lymphoama; FL, follicular lymphoma; HL, Hodgkin lymphoma.
**Leukemia:** ALL, actue lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; HCL, Hairy cell leukemia.

patients show only partial or brief B-cell depletion, while the clinical benefit, if any, is modest.[35] This failure suggests that SLE results from altered B-cell sensitivity, or possibly a complement (C) dysfunction.[36] Multiple sclerosis and antineutrophil cytoplasmic Abs-associated vasculitides have been studied for RTX efficiency, but controlled clinical trials have not yet been reported (Table 1).[37,38]

### AID suspected of answering Abs

Finally, anecdotal cases of anti-CD20 B-cell depletion have been reported in autoimmune-hemolytic anemia, cryoglobulinemia, acquired factor VIII-inhibitor disease, glomerulonephritis, pemphigus vulgaris, systemic sclero-derma, polymyositis and dermatomyositis.[39–43] An ongoing phase-I trial is even treating inactive psoriasis with RTX. Intriguingly, however, a handful of case reports suggest that this B-cell ablative therapy may actually induce psoriasis.[44,45]

## Graft rejection and graft versus host disease

The pathogenic role of B lymphocytes was highlighted in renal, cardiac, liver or pancreas transplantation, justifying B-cell depletion with RTX.[46–48] Allogeneic hematopoietic stem cell transplantation may induce acute or chronic destruction of the host by the donor's immune system. Whilst pathogenic mechanisms involved in these phenomena are not clearly understood, RTX therapy may be effective for some patients (Table 1).[49]

## Action mechanisms of rituximab
### Complement-dependent cytotoxicity

C may be utilized by RTX, and C-dependent cytotoxicity (CDC) seems to be pivotal in the efficiency of RTX.[50] In fact, after RTX treatment, C deficiency or consumption is strik-ing in patients with CLL,[51–53] with the over expression of C inhibitors CD55 and CD59.[18,22] Klepfish et al tried to enhance the activity of RTX in CLL by adding fresh frozen plasma in an attempt to correct the C imbalance.[52] Moreover, resistance to RTX therapy in NHL was ascribed to CDC inefficiency, as polymorphisms in the C1qA gene were affecting its clinical response and the duration of the response.[54,55] Following treat-ment with RTX, the CD20 molecules are clustered in the LRs, concentrating IgG Fc regions locally and therefore inducing fixation of C1q. The C molecule, C3, has been shown to vary in affinity for LRs by supplementing cholesterol in cellular membrane and by virtue of the hypersensitivity of CD55 to phospholipases.[56,57]

## Antibody-dependent cell-mediated cytotoxicity

ADCC occurs when B-cells are killed by monocytes, mac-rophages, natural killer (NK) cells and neutrophils following the binding of RTX to the Fc region. Among the Fc-gamma receptors (Fcγ), FcγRII was shown to inhibit ADCC, whereas FCγRIIIA behaved as an activator. However, a polymorphism of this latter receptor was also found to modulate ADCC. On the other hand, there is a dimorphism on residue 176 which can either be valine, with a high binding of IgG1, or pheny-lalanine with a low binding.[58] Cartron et al confirmed that a homozygous valine 158 polymorphism of FcγRIIIA leads to higher response rates and molecular complete remission rates in NHL patients after RTX treatment.[59] However, in CLL, ADCC was not influenced by the FcγR genotype expressed by autologous NK cells.[17]

## Apoptosis

RTX-induced apoptosis requires translocation of CD20 into the LRs.[60] This segregation increases cytosolic $Ca^{2+}$ levels through association between CD20 and the B-cell receptor (BCR).[61] Interaction between CD20 and raft mem-brane protein permits activation of components such as the phosphotyrosine kinases Lyn, Fyn, and Lck, as well as p75/80 (Cbp/PAG). This appears to be necessary for RTX,[57] downstream signals mediated by the mitogen-activated pro-tein kinase (MAPK) p38, cleavage of caspase-3, inhibition of Bcl-2, DNA fragmentation and, ultimately, apoptosis.[62] Growth inhibition of NHL cell lines by RTX may be caused by externalization, location in LRs with CD20 and acid sphingomyelinase production through ceramide synthesis, which also activates MAPK. However, Chan et al demon-strated that, in several cell lines at least, redistribution of CD20 was not crucial.[63]

## Why is the efficacy of RTX variable?
### Is the quantity relevant?
#### Number of lymphocytes

The efficacy of anti-CD20 mAb depends of the number of tumor cells. In fact, when the tumor burden is high, the vari-ous effector mechanisms engaged by RTX are saturated.[19] Kennedy et al confirmed this observation through an analysis of elevated B CLL cell concentration binding by RTX, which led to complete consumption of C lytic activity and depletion of several C components.[53] Their replacement with purified C proteins is sufficient to restore the cytotoxic activity of RTX. In aggressive DLBL, association between RTX and

chemotherapy improves the rate and increases the duration of the response.[64]

## Other variables for a fixed number of B-cells
### Number of CD20 molecules

CD20, a nonglycosylated phosphoprotein, is expressed in large quantities on the surface of almost all normal and malignant B-cells, up to 250,000 molecules per cell. However, resistance to RTX was identified in certain diseases, and especially in CLL. Mankai et al and others have established that this is related to a lower density of CD20 on B-cells in CLL compared to B-cells from other lymphomas,[65] associated with a down-regulation of the purine-rich box-1 (PU.1) transcription factor, so that transfection of B-cells with its cDNA restores RTX-induced lysis. Similarly, stimulation of B-cells with CpG increases the expression of CD20 and ameliorates the CDC by RTX.[66] The same results have been observed in lymphoma,[20] but Weitzman et al claim that there was no correlation between ADCC and CD20 density.[17]

### Location of the B-cells

After an infusion of RTX, circulating B-cells are rapidly removed. In contrast, germinal-center and marginal-zone B-cells resist this destruction.[67] This phenomenon seems to be linked to factors such as BAFF (B-cell-activating factor) from the microenvironment, integrins and the lack of vascularization, rather than to poor tissue penetration by RTX. This factor is more decisive for RTX susceptibility than for the density of Fcγ receptors on the membrane of all effectors.[68] We can note that the level of BAFF in sera from patients with lymphoma may be a useful indicator in predicting the efficacy of treatment with RTX and chemotherapy.[69] Furthermore, baseline serum levels of BAFF are inversely correlated with the induration of B-cell depletion after RTX treatment in primary Sjögren's syndrome.[70] These findings provide new options for treating AID and hematological malignancies.

## Quality implication
### Lipid rafts

LRs have been described as stable structures, 100–500 nm in diameter, which resist extraction by nonionic detergents at low temperature due to their high cholesterol and glycosphingolipid content.[71] This definition is misleading and may not apply to the composition of LRs *in vivo*. In fact, this structure is no longer accepted, and LRs are considered to be very heterogeneous, differing in the temporal stability of the composition of their lipids and proteins such as Src kinases.

The generally accepted definition of LRs was agreed at the 2006 Keystone symposium on LRs and cell function: "Lipid rafts are small (10–200 nm), heterogeneous, highly dynamic, sterol- and sphingolipid-enriched domains that compartmentalize cellular processes". The largest components of LRs are glycosphingolipids such as ganglioside M1 (GM1), sphingomyelin (SM), cholesterol and phosphatidyl serine, the last three in approximately 50% greater quantities than in plasma membranes (Figure 1). "Small rafts can sometimes be stabilized to form larger platforms through protein–protein and protein–lipid interactions".[72–74] When plasma membrane lipids and protein are clustered in the LRs, they can mediate transduction pathways which favor cellular adhesion, transmembrane signaling, virus budding, control of ionic pumps and channels, and mediation of vesicle fusion.[75,65]

Proteins connected to LRs have been described as insoluble in the nonionic detergent Triton X-100 at low temperature, in contrast to the other membrane lipids.[70] This means we should be careful when investigating proteins linked to rafts, as analyses of fluorescence microscopy or fluorescence resonance energy transfer confirm these data.[77] Many of these signaling proteins either reside in, or are transferred into, the LRs during signal transmission. Their fine-tuned regulation is probably influenced by nanoscale membrane microdomains, which are defined as dynamic and which apportion them to the membrane.[78] These include CD20,[79] BCR,[77] Fas, HLA II,[80] TNF receptor, CD40,[81] phosphatidyl inositol 3-kinase and several protein kinase C isoforms. Cbp/PAG, a ubiquitous, highly tyrosine-phosphorylated adaptor protein, only occurs in LRs, and plays an important part in the recruitment of Lyn, Fyn and Lck kinases into these LRs (Figure 1).[82]

Glycosphingolipids are involved in cell proliferation and differentiation. Glycosyl ceramides are the most widely distributed glycosphingolipids in cells, and act as precursors for the biosynthesis of many glycosphingolipids. GM1, widely used as a marker for LRs, is a sialoglycosphingolipid composed of a ceramide hydrophobic portion and a oligosaccharide sequence (Figure 2). It is integrated into the external leaf of the plasma membrane, and is at the forefront of the interaction of cells with their environment. For example, GM1 is known as a receptor for toxins and viral particles.[83,84] Furthermore, close interaction between SM and cholesterol is necessary for LR formation, and sphingomyelinase can transform small LRs into large, ceramide-enriched ones. In addition, glycosphingolipids can be metabolized into lysosphingolipids and sphingoid bases. This function as a metabolite acts as second messenger in signal transduction pathways of growth and apoptosis.[85,86]
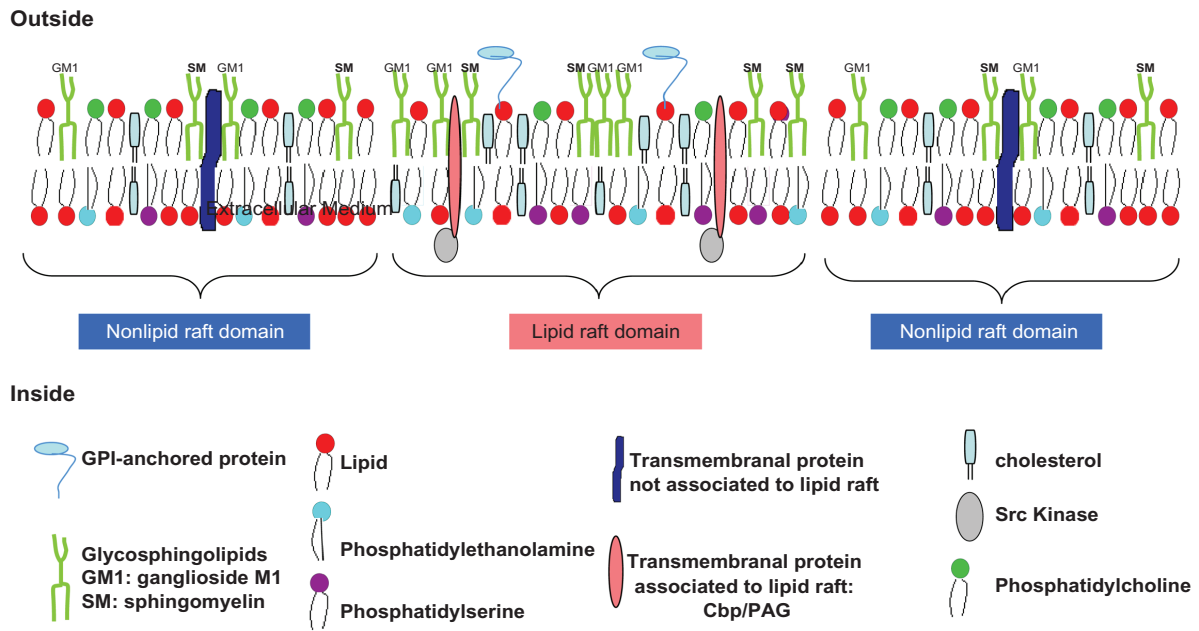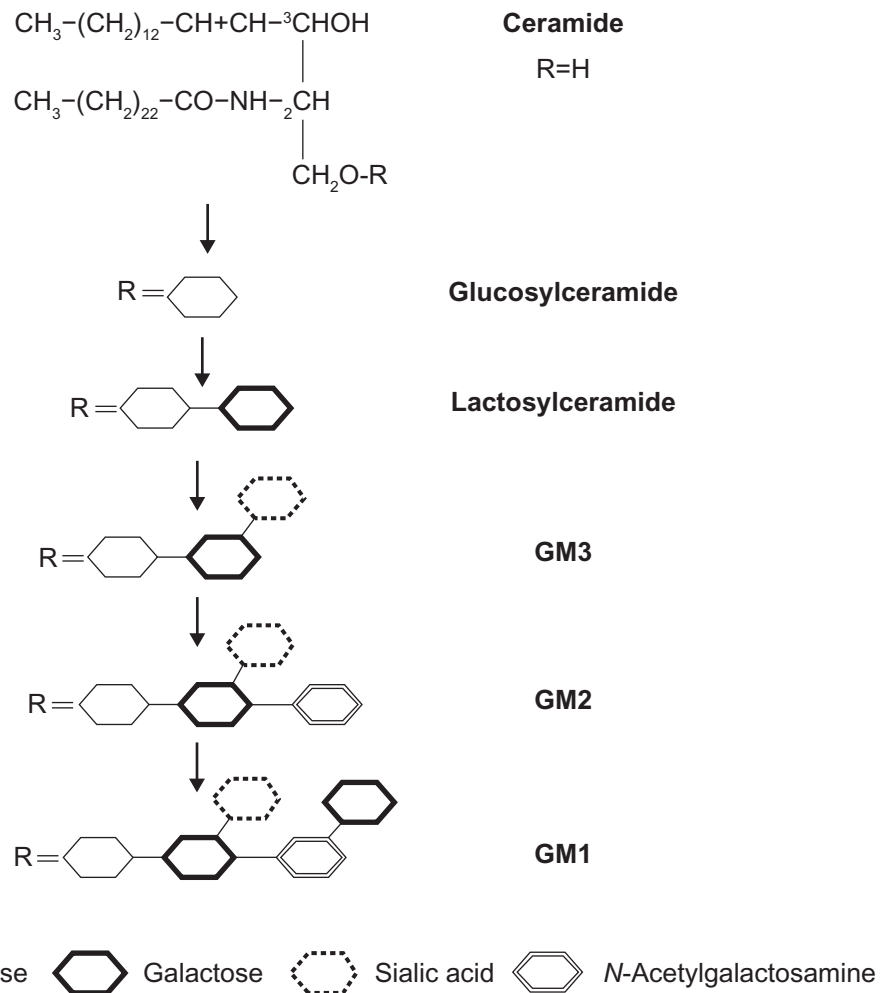
**Outside**

GM1    SM  GM1         SM    GM1 GM1 SM      SM GM1 GM1      SM  SM      GM1        SM GM1         SM

Extracellular Medium

Nonlipid raft domain          Lipid raft domain          Nonlipid raft domain

**Inside**

| | | | | | |
|---|---|---|---|---|---|
| | GPI-anchored protein | | Lipid | | Transmembranal protein not associated to lipid raft |
| | Glycosphingolipids GM1: ganglioside M1 SM: sphingomyelin | | Phosphatidylethanolamine | | Transmembranal protein associated to lipid raft: Cbp/PAG |
| | | | Phosphatidylserine | | |

cholesterol

Src Kinase

Phosphatidylcholine

**Figure 1** Lipid raft organization.

$$CH_3-(CH_2)_{12}-CH+CH-^3CHOH$$

$$CH_3-(CH_2)_{22}-CO-NH-_2CH$$

$$CH_2O-R$$

**Ceramide**

R=H

R = **Glucosylceramide**

R = **Lactosylceramide**

R = **GM3**

R = **GM2**

R = **GM1**

Glucose      Galactose      Sialic acid      *N*-Acetylgalactosamine

**Figure 2** Synthesis of ganglioside GM1 from ceramide.

## Variation in their composition

A recent study analyzing LR constitution in mantle cell lymphoma has revealed a downregulation of key LR proteins such as raftlin and Cbp/PAG.[87] These are involved in B-cell transduction and in the pathology of lymphoma. Moreover, the presence of Cbp/PAG in the LRs is necessary for RTX to effect transmembrane signaling.[57] The resistance of tumor B-cells to RTX may be explained by a deficiency in Cbp/PAG. If we take this a step further, changes in the expression of a number of glycosphingolipids on the cell surface have been correlated with typical cancer phenotype and tumor progression. In this respect, GM1 expression differs between lymphoma subtypes, even within one lymphoma subgroup. A recent study has demonstrated a correlation between the expression of GM1 and response to RTX in NHL and CLL.[88] In this latter leukemia, B lymphocyte membrane appears with increased fluidity, and produces lipid dynamics and lipid-protein interactions.[89,90] Furthermore, glucosylceramide, lactosylceramide and SM improve activity of P-glycoprotein, a membrane efflux transporter, which frequently underlies cancer cell and bacterial resistance.[91] There are excessive quantities of B lymphocytes in CLL patients.[92,93] In addition, glucosylceramide synthase inhibitors stimulate CLL cells to react to conventional cytotoxic and cytostatic drugs used in treatment.[94]

Finally, the disialoganglioside (GD3), a ganglioside weakly expressed in most normal tissues, is over-expressed during development and in pathological conditions such as cancer. It is responsible for a variety of events such as proliferation, differentiation and apoptosis.[95] However, the relationship between ganglioside GD3 and CLL is not fully understood. It should be noted that alterations in the LR localization of certain signaling molecules contribute to the acuteness of B-cell-dependent autoimmune disease in some cases. On the other hand, the majority of SLE patients exhibited a low level of Lyn expression as well as a diminished association with LRs, due to an increase in ubiquitination through translocation of c-Cbl into LRs.[96,97] This is associated with the abnormal expression of CD45, a membrane protein, tyrosine phosphatase, which controls Lyn expression by modulating its phosphorylation.[98] Lyn serves as a negative regulator for B-cell signaling, and reduced negative signaling in LRs hyperactivates B-cells, leading to uncontrolled production of IL-10 and auto-Abs. The development of SLE induced by abnormalities in the sub-cellular localization of the BCR signaling pathway was confirmed by the discovery of a single polymorphism in the FcγRIIB gene.[99] This mutation excludes FcγRIIB from the LRs and impedes its inhibitory effects on BCR signals.[100]

## Efficacy of other anti-CD20 mAbs

There is a clear need to develop new agents in order to improve the efficacy of B-cell depletion. In fact, there are many forms of resistance to RTX in autoimmune conditions and B-cell malignancies. On the other hand, other anti-CD20 mAbs have been, or are currently being developed (Table 2).

## CDC-improving anti-CD20 mAbs

Ofatumumab (2F2, HuMax-CD20, Genmab/GSK) is a fully-humanized, anti-CD20 mAb which binds to a portion closer to the B-cell membrane, and which is composed of the 74–80 amino acids in its small extracellular loop (Figure 3). Preclinical studies have thus far established that Ofatumumab has a slower rate of dissociation from CD20 than RTX and greater CDC power. Lysis of refractory RTX-resistant B-cells is also effected through this mAb,[50] with sustained success.[14] Moreover, infusion reactions which appear after use of Ofatumumab are similar to those which appear with RTX, and it is more effective in CLL patients.[101]

Veltuzumab (IMMU-106, hA20, Nycomed/Immunomedics) is a humanized mAb with complementarity-determining regions (CDRs) identical to RTX, except at position 101 in the CDR3 of the variable heavy chain (VH) in aspartic acid substitutes for asparagines, and in framework regions of Epratuzumab, another humanized anti-CD22 Ab. This difference induces ADCC identical to that of RTX in CLL.[17] There is a reduced off-rate[102] and greater efficacy in lysing tumor cells *in vitro* by CDC, and also *in vivo* with a phase I/II for recurrent/refractory NHL.[13]

## ADCC-increasing anti CD20 mAbs

Ocrelizumab (2H7) is a 90%–95% humanized mAb (Genentech/Roche/Biogen-Idec), and binds to an epitope which is different to that of RTX but which overlaps with it, and which is in the extracellular domain of CD20.[103] Because C activation leads to side effects associated with RTX, a modification of its Fc portion is necessary. Its gives mAb a reduced CDC[12] and increases its tolerability in autoimmune diseases. On the other hand, ADCC is higher than with RTX, due to amplified binding affinity for the low-affinity variants of FcγRIIIa.

Modifications to anti-CD20 Ab glycosylation, aimed at increasing ADCC, have generated three anti-CD20 mAbs. GA-101 is a humanized third-generation and glyco-engineered version of anti-CD20 mAb (Glycart Biotechnology AG, Genentech Inc, F Hoffmann-LaRoche Ltd, Biogen Idec Inc and Chugai Pharmaceutical Co Ltd) which produces greater ADCC, superior direct cell death and greater efficacy in depleting B-cells than RTX in the potential treatment of NHL or CLL.[104] EMAB-6, with a low fucose content, triggers similar apoptosis and

**Table 2** Major characteristics of anti-CD20 antibodies compared to activity of rituximab

| | Antibody Specificity | Mechanisms of action | | | | |
|---|---|---|---|---|---|---|
| | | CDC | ADCC | Apoptosis | B cell depletion | Very efficient in B cells expressiong low CD20 levels |
| Ofatumumab (2F2-Genmab/GSK) | fully human IgG1 | +++ | | ++ | | + |
| Veltuzumab (IMMU-106, hA20-Nycomed/Immunomedics) | humanized IgG1 | +++ | | ++ | ++ | |
| Ocrelizumab (2H7-Genentech/Roche/Biogen-Idec) | humanized IgG1 | +/− | ++ | + | | |
| GA-101 (Glycart Biotechnology AG, Genentech/Roche) | humanized IgG1 | − | +++ | +++ | ++ | |
| EMAB-6 (LFB) | chimeric with low fucose content | + | ++ | + | | + |
| BLX-301 (Biolex/Argen) | humanized | | ++ | | ++ | |
| Experimental mAb 1,5,3 (Amgen/Astrazeneca) | fully human | + | + ++ (FcγRIIIa) | ++ | ++ | |
| Tositumomab (B1-GlaxoSmithKline) | mouse IgG2a | − | +/− | ++ | | |

**Abbreviations:** CDC, complement-dependent cytotoxicity; ADCC, antibody-dependent cellular cytotoxicity.
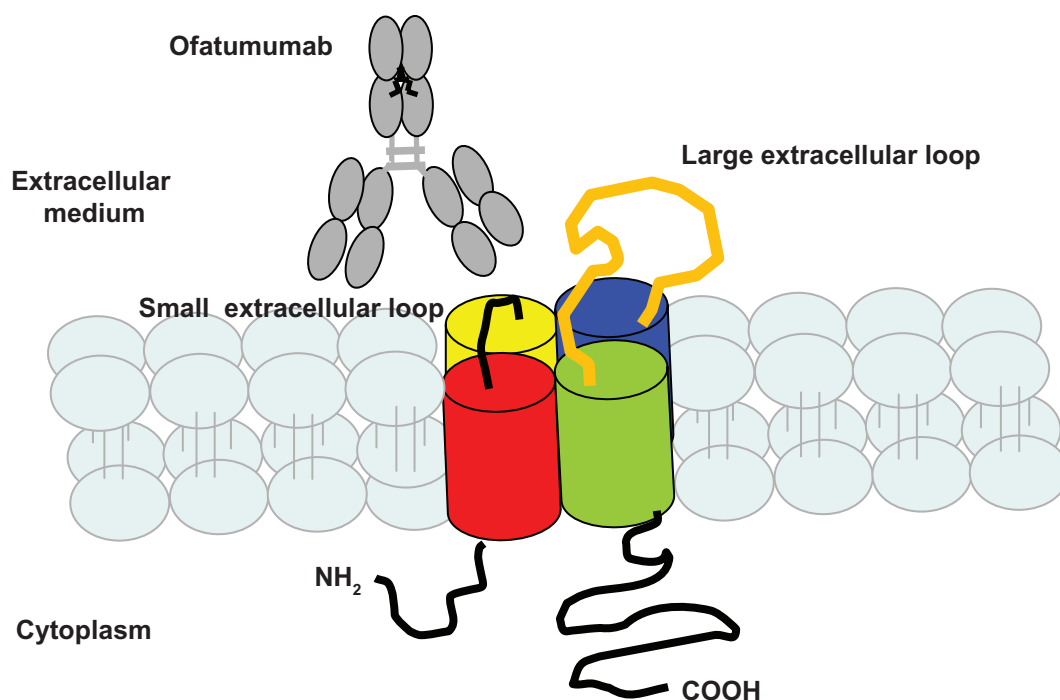


**Figure 3** Fixation of ofatumumab on CD20 small extracellular loop.

CDC to that observed for RTX, but promotes ADCC in CLL. The different levels of efficacy are more pronounced with low doses and when target cells express fewer CD20 molecules.[105] BLX-301 (Biolex/Aragen) is a humanized anti-CD20 mAb with an optimized glycosylation structure, enhanced ADCC, potent B-cell depletion, and potentially low side effects. BLX-301 is being developed for treatment of NHL.

Finally, the objective of protein engineering is to manufacture an anti-CD20 Ab with greater potency and efficacy in all patients. Thus, vaccines are being developed with CD20 mimotope peptides[106] and AME-133v (Lilly), which improve the binding of anti-CD20 mAb to the Fc receptor on their immune effector cells.

## Anti-CD20 mAbs which increase programmed cell death

Zevalin, an anti-CD20 mAb linked to Yttrium-90 (IDE-CY2B8, Ibritumomab Bayer Biogen Idec) and Bexxar (Tositumomab, B1 coupled to iodine I131-GlaxoSmithKline) were approved by the FDA in 2002 and 2003 respectively for treatment of NHL and for trials in other malignant diseases such as CLL.[107] It is worthy of note that unradiolabeled tositumomab was described as inducing stronger apoptosis and ADCC than RTX.[108]

The Amgen/AstraZeneca mAb 1.5.3 better enhances proapoptotic activity *in vitro* than RTX, and mediates both CDC and ADCC, with superior ADCC when NK donor cells present an FcγIIIa F/F allotype. In a primate pharmacodynamic model, this promotes higher B-cell depletion in lymph node organs and BM.[109]

The genetically-engineered tetravalent Ab (TetraMcAb), derived from the anti-CD20 mAb RTX and ofatumumab, exhibits more potent antiproliferative and apoptosis activities than ofatumumab or RTX.[110] Hex-hA20, which comprises six Fabs with one Fc, translocates CD20 in LRs, affects ADCC but not CDC, and inhibits proliferation of NHL cell lines *in vitro* at low level concentration without requiring antibody cross-linking.[111]

## Conclusion

The efficacy of anti-CD20 mAbs seems to depend, at least in part, on their ability to translocate CD20 molecules into LRs. Apoptosis is higher in those which cannot do so, where mAbs which can do so are characterized by CDC. ADCC is the same in both types. New anti-CD20 mAbs have been developed based on this classification. Mechanisms differ according to the type of B-cell-disease and the type of patient. These mAbs will be able to be used to best effect when their precise roles are known, and when B-cell-disorder physiopathology is better understood. For the time being, performance could be improved by increasing doses of mAbs, changing the type of mAb for each disease, associating different drugs simultaneously or successively, and modifying the LRs.

## Disclosure

The authors report no conflicts of interest in this work.

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