Review

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CD20-depleting therapy in autoimmune diseases: from basic research to the clinic

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The B lymphocyte-associated antigen CD20 is becoming an important immunotherapy target for autoimmune diseases, although its biological function has not been defined. Besides rheumatoid arthritis, growing experience with B cell-depleting therapy indicates that it may be effective in Sjögren's syndrome, dermatomyositis–polymyositis, systemic lupus erythematosus and some types of vasculitides. However, controlled clinical trials are still lacking for some of these indications. Infection has not been seen as a major limitation to this therapy, but reports of progressive multifocal leukoencephalopathy in an extremely small number of patients are of concern. Here, we review the therapeutic actions of anti-CD20 antibodies, and the recent and ongoing clinical trials with CD20-depleting therapy in autoimmune diseases.

Keywords: autoimmune diseases, CD20, immunotherapy, monoclonal antibodies, target antigen.

Introduction

The introduction of monoclonal antibody (mAb) methodology by Köhler and Milstein almost 35 years ago [1] generated great enthusiasm within the scientific community at the prospects of using these reagents to target specifically pathological cells and soluble factors in novel immunotherapies. Despite much initial effort, it was not until 1986 that a therapeutic application of mAbs emerged, with the approval in the United States of the anti-OKT3 mAb muronomab for use following transplantation (http://www.accessdata.fda.gov/Scripts/cder/DrugsatFDA/index.cfm). Another early clinical application was the development of custom-made anti-idiotype mAbs, first reported in 1985, to treat patients with low-grade B cell lymphoma [2]. As muronomab and the anti-idiotype mAbs were entirely murine, they were immunogenic and induced an immune response in patients, thus limiting their use. Moreover, tailoring mAbs to patients' idiotypes was costly, time-consuming and not as effective as anticipated [3]. Nonetheless, the commercial launch of muronomab, at a time of high expectations for the therapeutic potential of mAbs, supported the further development of this branch of biotechnology. Over the next few years, there was tremendous progress in cellular and molecular biology, leading to the identification of new molecules as potential targets of immunotherapy, as well as in biotechnology, resulting in the production of safer and more efficacious antibodies of higher human composition (chimeric, 'humanized' and fully human mAbs).

The first chimeric (murine–human) mAb approved by the US Food and Drug Administration (FDA) for the treatment of autoimmune diseases was infliximab. This anti-tumour necrosis factor (TNF)-α mAb was approved for the treatment of Crohn's disease in 1998 and rheumatoid arthritis (RA) in 1999. In 2006, the chimeric mAb rituximab was approved for the treatment of RA; this anti-CD20, B cell-depleting mAb had already been authorised for the treatment of B

cell tumours in 1997. From these initial experiences with immunotherapy of autoimmune diseases, two major points emerged. First, an effective therapeutic mAb can have a tremendous impact on the natural history of the disease. Second, much remains to be discovered about the pathogenesis of rheumatic diseases and the role of B lymphocytes in sustaining the autoimmune process. How B cell depletion ameliorates the symptoms of RA is unclear, especially as T cells have been considered to be the main cause of synovial damage, and is currently the subject of investigation.

In view of the increasing number of autoimmune diseases that appear to respond to B cell depletion, here we review the recent and ongoing clinical trials with CD20-depleting therapy in autoimmune diseases. We focus on rituximab, which is the most used clinically with a wealth of information about its *in vitro* effects. The newly synthesized humanized and fully human anti-CD20 mAbs will be only briefly discussed, although it is expected that these mAbs will provide a higher therapeutic index and greater efficacy [4]. First, we review the structure and function of CD20 along with the mechanisms that mediate the therapeutic effects of anti-CD20 mAbs. Second, the clinical trials of CD20-depleting therapy for autoimmune diseases are discussed. Multiple sclerosis and immunoglobulin (Ig)M-mediated neuropathy are not considered as they have been recently reviewed elsewhere [5, 6]. Finally, we consider rituximab-related adverse events, in particular bacterial and viral infections, which are prompting the medical community to reappraise the safety of biological therapy in autoimmune diseases.

CD20: structure, function and rationale for use as a target of nonselective immunotherapy

CD20 structure

CD20 is a 33-kDa protein expressed by mature B cells and most malignant B cells, but not by preB mature or differentiated plasma cells. Although its three-dimensional structure has not been defined, its amino acid sequence predicts a tetra-spanning integral membrane protein belonging to the MS4A family, with intracellular C- and N-termini and two extracellular loops of nine and 43 residues spanning from positions 72 to 89 and from 142 to 184, respectively [7, 8].

CD20 can co-exist in different forms in the plasma membrane, depending on the amount of cholesterol present [9] and on the intensity of its association with raft microdomains [10, 11]. Within the rafts, CD20 has been found to be associated with CD40 and MHC class II antigen [12]. The functional significance of these physical associations is unclear.

Exploring CD20 function with artificial ligands

Inasmuch as the natural ligand of CD20 – if indeed there is one – is unknown, many of the functions of CD20 have been identified using artificial ligands (mAbs). *In vitro* studies employing panels of anti-CD20 mAbs have revealed that CD20, through its ability to associate with the B cell receptor [13], acts as an ion channel, in particular as a calcium channel [14], and may also activate intracellular signalling, leading to cell cycle arrest, homotypic adhesion, apoptosis or lysosome-mediated cell death [15–18], depending on the specific epitope bound by the mAb.

The particular CD20 epitopes bound by different mAbs have been identified using site-directed mutagenesis [19] and phage display peptide libraries [20, 21]. From such studies, it is evident that most anti-CD20 mAbs characterized so far, including rituximab, bind to the larger 43-residue extracellular loop, particularly in the area between A^{170} and P^{172} [19, 20, 22]. However, despite interacting with a restricted area of CD20, these mAbs induce a range of effector functions, including apoptosis, homotypic aggregation and complement activation. Thus, anti-CD20 mAbs have been classified into two groups: (i) type I mAbs (e.g. rituximab and 1F5) strongly activate complement and trigger effector functions, but are poor inducers of apoptosis and homotypic aggregation; (ii) type II mAbs (e.g. B1) are poor activators of complement but strong inducers of homotypic adhesion, apoptosis and/or lysosome-mediated cell death [16–18]. Further heterogeneity has recently been outlined for the type I

mAbs rituximab and 1F5 in experiments using peptide mimotopes of CD20 epitopes recognized by rituximab: a cyclic 7-mer peptide bearing the rituximab-specific CD20 motif <ANPS>, homologue to the ^{170}ANPS^{173} portion of CD20, was also recognized by 1F5 whilst a 12-mer linear peptide whose sequence did not match any portion of the extracellular loop of CD20 was not bound by 1F5 [20]. These data suggest that rituximab has a unique specificity for CD20 and may also recognize a second portion of the cell surface antigen [23]. It remains to be determined whether this additional specificity is responsible for the relatively low rate of cellular internalization of bound rituximab (compared with 1F5) [24] or for the ability of rituximab to reverse multidrug resistance (not seen with 1F5) [25], and also whether it is important for the therapeutic efficacy of rituximab.

Rituximab treatment of autoimmune diseases: poor scientific rationale but golden therapeutic opportunity

Rituximab is a chimeric mAb derived from the mouse mAb 2B8 following replacement of the heavy and light chain constant regions with the corresponding regions of a human IgG1 mAb. The rationale for use of rituximab in the first pilot (phase I) clinical trials was poor for several reasons. First, there was a lack of data from animal models. Second, the role of CD20 in B cell maturation and differentiation was unclear, particularly because these functions were unaffected in CD20 knockout mice [26]. Third, no ligand for CD20 had been identified at the time, and it is still unclear whether this is a cell surface receptor. Finally, as CD20 is not selectively expressed by autoreactive B cells, there was concern that general depression of the immune system could be induced in autoimmune patients, despite the fact that there was no indication of enhanced risk of opportunistic infections in non-Hodgkin's lymphoma patients treated with rituximab.

However, two molecular features of CD20 made it an attractive target for immunotherapy: (i) it does not internalize upon mAb binding; and (ii) it is not shed from the cell surface. These are ideal properties for a therapeutic mAb in order for its effector functions to be fully activated in the target cell.

The mechanism of B cell killing by rituximab and other anti-CD20 mAbs and the factors influencing the potency of this activity have been exhaustively reviewed [4]. Briefly, the mechanism depends on the effector functions of the mAb [i.e. antibody-dependent cell-mediated cytotoxicity (ADCC) (or complement-dependent cytotoxicity)] [8] and/or on its unique epitope specificity, which influences its ability to induce apoptosis [27], inhibit proliferation, and/or activate acid sphingomyelinase [28] (Fig. 1). Currently, it is believed that ADCC is the major mechanism of action of rituximab-induced B cell depletion. The effector functions of rituximab have represented the main rationale behind its initial use in those autoimmune diseases clearly triggered by pathogenic auto-antibodies, including cold agglutinin diseases [29] and idiopathic immune-mediated thrombocytopenic purpura (ITP) [30]. Even so, these mechanisms do not entirely explain the therapeutic effects of rituximab in RA.

B cell function in autoimmune diseases: mechanism of action of rituximab

The dogma that B cells are simply passive recipients of signals necessary for their differentiation into autoantibody-producing plasma cells was challenged with the early demonstration by Lanzavecchia that B cells behave as efficient antigen-presenting cells [31]. Subsequently, a number of studies have highlighted other functions of B cells [32, 33], including the ability to promote T cell accumulation and activation in the MLR/lpr mouse [34], to mediate antibody-independent autoimmune damage [35], and to provide co-stimulatory molecules [36] and cytokines (e.g. TNF-α, interleukins-4 and -10) that sustain T cell activation in rheumatoid synovium [37]. All these functions, which are clearly suggestive of a broader role played by B cells in the pathogenesis of autoimmune diseases, can be neutralized by rituximab-induced B cell depletion.

Phenotypic analysis of the B cells that repopulate the blood after rituximab treatment provided further insight into the mechanism of action of this mAb [38]. In a long-term follow-up of systemic lupus erythematosus (SLE) patients treated with rituximab, the

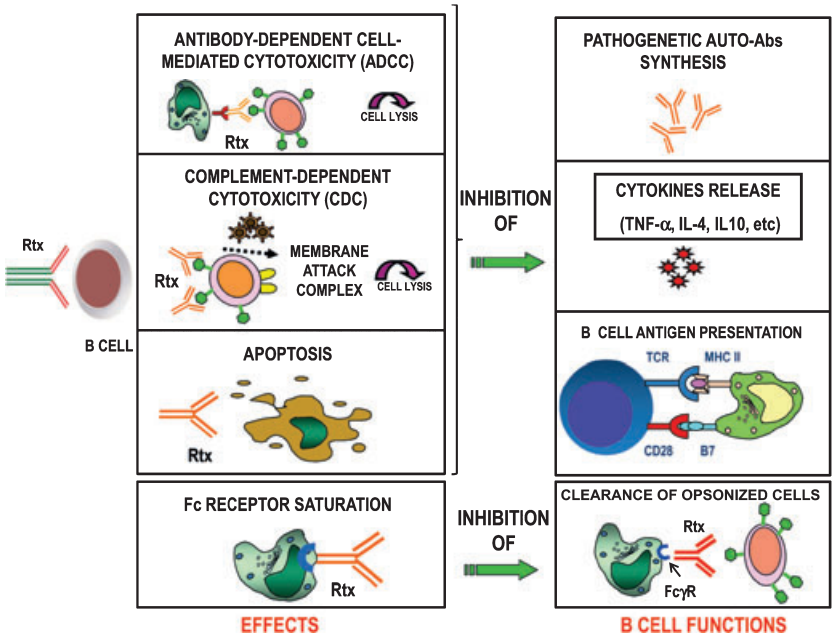

Fig. 1 Proposed mechanisms of action of rituximab (Rtx) and consequences on B cell function.

predominant phenotype of B cells at reconstitution was $CD5^{+}CD38^{+}$ (transitional cells), whereas there was few switched memory B cells ($CD27^{+}$). This B cell profile resembles that seen during normal ontogenesis as well as during repopulation after bone marrow transplantation, and suggests some kind of immune system resetting. It is interesting that repopulation of $CD27^{+}$ B cells occurred several months after that of $CD27^{-}$ B cells.

Recently, a simple mechanism of action has been proposed for rituximab that is similar to that triggered by hyperimmune anti-RhD IgG in the treatment of ITP. According to the immune complex decoy hypothesis, rituximab acts by saturating the Fc receptors of effector cells, thereby protecting opsonized platelets from clearance by the saturated FcR-bearing effector cells (Fig. 1) [39]. There is currently no experimental evidence to support or dispute this hypothesis.

The first clinical evidence of the mechanism of action of rituximab in T cell-mediated diseases came from a patient with both B cell lymphoma and RA [40]. Rituximab treatment, indicated for lymphoma, effectively induced remission of the arthritic symptoms without reducing the serum levels of a concomitant IgM monoclonal component (expression of tumour bulk). These observations opened the way for treatment of other patients with RA and autoimmune diseases such as SLE, Sjögren's syndrome (SS), anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitides and multiple sclerosis (reviewed in [5, 41]).

Another remarkable demonstration of the action of rituximab, independent of the presence of pathogenic antibodies, was the rapid clinical response of two patients with both ITP and common variable immunodeficiency [42, 43]. Despite the heterogeneity of this second disease, patients usually have normal numbers of B lymphocytes but low or undetectable levels of IgG (and sometimes IgM and IgA levels too). Infusion of rituximab increased platelet counts despite the fact that these patients were refractory to conventional therapy for ITP [42, 43].

Clinical trials in rheumatological autoimmune diseases

Rheumatoid arthritis

Three controlled clinical trials [44–46] have demonstrated the efficacy of rituximab in patients with RA,

including those unresponsive to TNF-α inhibitor therapy [46]. Based on these studies, the European Medicines Agency (EMEA) established recommendations regarding dosage, duration of infusion and contraindications, and also summarized the common side effects of this treatment (Fig. 2) [47]. In particular, the recommended dose is 1 g, to be given once and then repeated 14 days later; this 'RA-like schedule' is different from that recommended for lymphoma (the 'lymphoma-like schedule', i.e. 375 mg m^{-2} in 4 weekly treatments). Based on these studies, and on the wide experience of the use of rituximab in lymphoma patients, the EMEA did not recommend pretreatment screening for tuberculosis but did recommend screening for hepatitis C (HCV) and B (HBV) virus, followed by lamivudine prophylaxis in HBV-positive patients. The authors of the consensus statement [47] acknowledged that rituximab treatment of RA was not curative and that retreatment was possible; however, it was not clear how many times patients could be retreated for relapses of this chronic disease.

Insight into developing an effective retreatment protocol came from a pilot study by Popa *et al.* [48] in which 37 patients received up to five cycles of rituximab therapy and were observed for up to 5 years. Rituximab was withdrawn in 15 patients (40.5%), because of the brevity of the response (eight patients), tachyphylaxis (five patients), infusion reaction (one patient) or respiratory complications (one patient). In the 22 responding patients, the mean duration of response per cycle was 15 months and the mean time to retreatment was 20 months. Indications for retreatment were the return of circulating B cells to normal levels, a clinical exacerbation or an increase in C-reactive protein concentration in the presence of adequate levels of immunoglobulins [48].

The first three clinical trials on which the consensus statement [47] is based, together with the retreatment trial [48], demonstrate that about 50% of RA patients who receive rituximab achieve a clinical response in terms of American College of Rheumatology 20% improvement criteria (ACR20) at 24 weeks and, amongst the responders, 50–60% can benefit from further infusions. However, none of these studies identified baseline factors predictive of the response to rituximab [49]. The only known predictive factor is a partial depletion of B cells during treatment, which is associated with a poor outcome [50].

Sjögren's syndrome

This connective tissue disease is pathologically similar to RA in three main ways. First, about 70% of

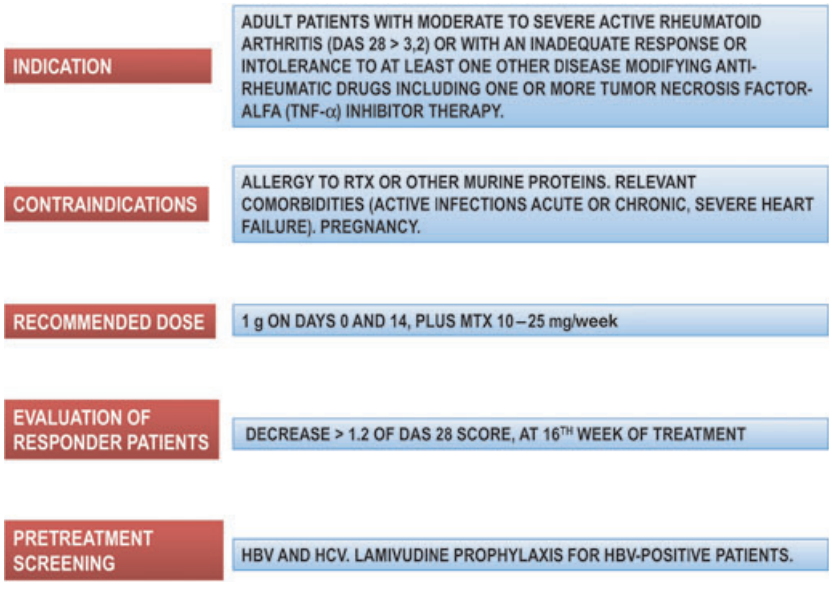

Fig. 2 Consensus statement recommendations for the clinical use of rituximab in rheumatoid arthritis [47]. *DAS28*, disease activity score measured in 28 joints.

patients with SS are rheumatoid factor (RF) positive. Second, inflammatory arthritis in SS is common (although without erosions). Third, some RA patients have xerostomia and xerophthalmia, which are characteristic of SS. The fortuitous way in which the efficacy of rituximab was discovered in the two conditions is also similar: two patients treated with rituximab for marginal zone lymphoma had improvements in their symptoms of lymphoma-associated SS [51, 52]. To date, 66 patients with SS are reported to have been treated with rituximab (Table 1) [53–62].

One early open-label study administered four weekly injections of rituximab at 375 mg m^{-2} (lymphoma-like schedule) to 15 patients with primary SS, including seven with mucosa-associated lymphoid tissue (MALT) lymphoma [54]. The response rate was 73% (11 of 15 patients), although three of the patients without lymphoma discontinued treatment because of serum sickness from human anti-chimeric antibodies (HACAs). In a subsequent trial, 16 patients with primary SS received two rituximab infusions 1 week apart; a significant improvement in systemic symptoms was recorded at weeks 12 and 36 [62]. Another retrospective study of 16 women with primary SS, who subsequently developed nonHodgkin's lymphoma (five patients), mixed cryoglobulinaemia (five patients), polysynovitis (four patients) or mononeuritis multiplex (two patients), reported an improvement in sicca syndrome-associated dryness in 13 patients (81%) [61]. Finally, a double-blind, placebo-controlled trial was conducted recently to determine the efficacy and safety of an RA-like dosage (1 g on days 0 and 14) in 17 patients with primary SS (nine in the placebo group) [53]. The results demonstrated a significant improvement in clinical symptoms related to fatigue (measured on a visual analogue scale; VAS) in the rituximab group at 6 months (compared to baseline) as well as significant differences in SF-36 Healthy Survey scores between the rituximab and placebo groups at 6 months.

Overall, in these preliminary studies, rituximab treatment led to a reduction in RF (IgM) concentration in most patients without affecting the levels of anti-Ro/SSA and anti-La/SSB antibodies (IgG) [63]. Serum sickness reaction was the most frequent adverse event, which occurred mostly in patients who developed HACAs. Although these trials showed some benefits of treatment with rituximab for SS, the evidence is limited by the small study size and inadequate design, in particular regarding the enrolment of heterogeneous groups of patients with primary and

Table 1 Clinical trials with rituximab in patients (pts) with Sjögren's syndrome (SS) and dermatomyositis/polymyositis (DM/PM)

Disease	No. of pts in each study [reference][a]	Total no. of pts	Rituximab dose; no. of infusions; timing	Overall response (%)[b]	Adverse events (%)
SS	15 [54]; 1 [55]; 1 [56]; 6 [57]; 1 [58]; 1 [59]; 1 [60]; 16 [61]; 16 [62]	58	375 mg m^{-2}; 2–4; weekly	44–83	HACA-mediated serum sickness (9); infections (2)[c]
	8 [53]	8	1 g; 2; days 1 and 15	100	Serum sickness (12)
DM/PM	7 [64]; 4 [65]; 3 [66]; 4 [67]	18	100–375 mg m^{-2}; 4; weekly	75–100	None
	3 [68]; 8 [69]	11	1 g; 2; days 1 and 15	38–80 (muscle strength) 0 (skin lesions)	None
	1 [70]	1	1 g; 3; days 1, 15 and 30	NR[d]	None

[a]Noncontrolled studies or case reports.
[b]Complete plus partial responses.
[c]Human anti-chimeric antibody.
[d]Not reported.

secondary SS [57, 61] and the lack of a disease activity scoring system. Therefore, the role of CD20-targeting therapy in this clinical setting remains to be established in controlled clinical trials.

Two ongoing randomized, double-blind, placebo-controlled clinical trials designed to evaluate the efficacy of rituximab in primary SS have been registered at the clinical trials repository of the US National Institutes of Health (NIH) (http://www.clinicaltrials.gov). The main aim of the first study (NCT00426543, registered in 2007) is to evaluate the effects of B cell depletion on oral and ocular dryness and constitutional symptoms such as fatigue and myoarthralgia; currently up to 22 patients are being recruited for this phase II study. The primary end-point of the second trial (NCT00740948, registered in 2008) is the effect of rituximab on disease activity, measured as a 30% improvement in VAS score from day 1 to week 24; it is intended that this phase III multicentre study will enrol 120 patients by the end of 2009.

Dermatomyositis and polymyositis

Only a few, noncontrolled studies on the efficacy of rituximab for the treatment of dermatomyositis (DM) and polymyositis (PM), involving a total of 30 patients, have been reported (Table 1) [64–70]. Overall, these studies showed: (i) some improvement in muscle strength but only marginal benefit in cutaneous symptoms; (ii) short-term efficacy; and (iii) no clear optimal therapeutic dose (100 mg vs. 375 mg) or schedule for retreatment in refractory patients. A 52-month period of remission was reported in only one patient with DM after treatment with three courses of rituximab using an RA-like schedule; remission persisted even after the B cell level returned to normal [70].

One randomized, double-blind, placebo-controlled study has been registered with the NIH. This phase II study (NCT00106184, registered in 2005) will assess the efficacy of rituximab and the time to achieve improvement in patients with adult and juvenile DM and adult PM, over a period of 44 weeks. To date, 202 patients have been enroled.

Systemic lupus erythematosus

A number of different mechanisms have been postulated to explain the beneficial effects of rituximab in patients with SLE: (i) removal of autoreactive B cell clones with restoration of normal immune tolerance; (ii) reduction of auto-antibody levels, in particular anti-double-stranded DNA (anti-dsDNA); and (iii) synergism with cyclophosphamide [71]. As the first pilot study in 2002 that evaluated the safety and efficacy of rituximab in patients with SLE [72], a total of 192 patients have been treated in 12 studies (Table 2) [71–82]. The regimens most often used have been: (i) the lymphoma-like schedule; (ii) the RA-like schedule; and (iii) four 500-mg m^{-2} weekly infusions. Most studies confirmed the beneficial effects of the drug, especially in patients with renal and neuropsychiatric involvement. The overall response rate (complete plus partial responders) ranged from 78% to 90% and none of the schedules emerged as better than the others. However, in some studies [72, 73, 76], a clinical response was recorded with no significant changes in anti-dsDNA antibody or complement levels. Only baseline levels of serum C3 and anti-extractable nuclear antigen antibodies predicted relapse [83]. Treatment was generally well tolerated, although infectious adverse events were reported in two of seven patients with lupus nephritis [80] and in five of 10 patients with neuropsychiatric SLE [79]. Moreover, in a study of 22 patients with renal involvement, one patient died from invasive histoplasmosis [76].

Several conclusions can be drawn from these 12 studies of rituximab treatment for different types of SLE. First, rituximab is effective in children and does not interfere with growth or development [84]; severe adverse events were only reported in one study of 11 children (two cases of septicaemia and four of haematologic toxicity) [77]. Second, rituximab is also effective in neuropsychiatric SLE, inducing a rapid improvement in neurological signs and symptoms [79]. Finally, rituximab is effective in lupus nephritis, producing good clinical and histological responses [80]. However, data regarding the long-term efficacy of rituximab in SLE are limited. In one study, relapse occurred in seven of 11 SLE patients treated with rituximab using a lymphoma-like schedule; median

Table 2 Clinical trials with rituximab in patients (pts) with systemic lupus erythematosus (SLE)

No. of pts (clinical subset)	Rituximab dose; no. of infusions; timing	Concomitant immunosuppressive therapy	Overall response (%)[a]	Adverse events (%)	Reference
6	500 mg; 2; days 1 and 15	Cyc and GC	83	None	[72]
18	100 mg m^{-2}; 1 375 mg m^{-2}; 1 375 mg m^{-2}; 4; weekly	None	61	Infections (11)	[73]
24	1 g; 2; on days 1 and 15 500 mg; 2; days 1 and 15	Cyc and GC	96	Reversible pancytopenia (4)	[74]
7 (paediatric)	750 mg m^{-2}; 2, days 1 and 15	Cyc and GC	100	None	[75]
22	0.5–1 g; 2; days 1 and 15	Cyc, GC, Aza, Mtx, Mmf	90	Death (4)	[76]
11 (8 paediatric nephritis)	350–450 mg m^{-2}; 2∼12; weekly	Cys and GC	75	Septicaemia (18) Haematologic toxicity (36)	[77]
11	375 mg m^{-2}; 4; weekly	Cyc	100	None	[78]
16	500 or 1000 mg m^{-2}; 2; weekly	None	100	None	[71]
10 (neuro-SLE)	375 mg m^{-2}; 2; days 1 and 15 500 mg; 4; weekly 1000 mg; 2; days 1 and 15	None	100	Infections (50)	[79]
7 (nephritis)	375 mg m^{-2}; 4; weekly	Cyc and GC	100	Infections (28)	[80]
15	500 mg m^{-2}; 4; weekly 1000 mg; 2; days 1 and 15	None	60	None	[81]
45	1000 mg m^{-2}; 2; days 1 and 15	Cyc and CG	89	None	[82]

[a]Complete plus partial responses.
Cys, cyclophosphamide; GC, glucocorticoids; Aza, azathioprine; Mtx, methotrexate; Mmf, mycophenolate mofetil.

time to relapse was 12 months and retreatment using an RA-like schedule was successful [78].

Two ongoing multicentre, randomized, double-blind, placebo-controlled trials have been registered with the NIH. The EXPLORER trial (NCT00137969, registered in 2005) is a 52-week evaluation of the efficacy of rituximab in 250 patients with severe SLE. The LUNAR trial (NCT00282347, registered in 2006) is evaluating the efficacy of rituximab in combination with mycophenolate mofetil in 140 patients with stage III or IV lupus nephritis.

Vasculitides

Mixed cryoglobulinaemia. The aim of B cell depletion in mixed cryoglobulinaemia (MC) is to reduce IgM RF synthesis and arrest the proliferation of B cell clones that sustain the disease [85, 86]. As the first report of the use of rituximab in a man with refractory MC [87], seven off-label clinical trials involving 74 patients have been reported (Table 3) [87–93]. In a recent systematic review of the 57 MC patients treated with rituximab up to 2007, a clinical response was found in 80–93% of cases and a relapse in 39% of responders [94]. The majority of patients had received a lymphoma-like regimen of rituximab and tolerated the treatment well. Responders had improvements in skin lesions, neuropathy, arthralgia and renal function, a reduction in cryocrit and RF levels, and a normalization of serum C4 levels [90, 92, 95]. However, hepatitis C viral load increased in responders without substantially changing in nonresponders [90, 92].

Because of the observed increase in viral load during rituximab treatment in patients with HCV-related MC,

Table 3 Clinical trials with rituximab in patients (pts) with mixed cryoglobulinemia (MC) and ANCA-associated vasculitides

Disease	No. of pts in each study [reference]	Total no. of pts	Rituximab dose; no. of infusions; timing	Overall response (%)[a]	Adverse events (%)
MC	1 [88]; 4 [87]; 15 [89]; 20 [90]; 16 [91]; 12 [92]	68	375 mg m^{-2}; 4; weekly	80–100	None
	6 [93]	6	250 mg m^{-2}; 2; weekly	80	None
ANCA-associated vasculitides	9 [98]; 11 [99]; 11 [78]; 10 [100]; 11 [101]; 8 [102]; 8 [103]; 6 [104]; 7 [105]; 15 [106].	96 (WG, 80; MP,14; CSS, 2)	375 mg m^{-2}; 4; weekly	37.5–100	Infections (14); death (1)

[a]Complete plus partial responses.
WG, Wegener's granulomatosis; MP, microscopic polyangiitis; CSS, Churg-Strauss syndrome.

two new protocols have been introduced. The first combines rituximab with anti-viral therapy (pegylated-interferon and ribavirin) [96]. Of 16 patients with HCV-related MC treated with this protocol, 15 (94%) had some clinical and virological response, including 10 (63%) with a complete response. At a mean follow-up of 19.4 months, two responders (13%) had relapsed, with reappearance of HCV RNA and cryoglobulinaemia and a return of B cells to normal levels. The second protocol involves administering a lower dose of rituximab (250 mg m^{-2} rituximab on days 1 and 8) [93]. In a pilot study in six patients with HCV-related MC, this schedule led to a complete clinical and laboratory response in four of five evaluable patients (one patient died); the one nonresponder did not benefit from retreatment with two further doses of rituximab at 375 mg m^{-2}. This protocol was as effective as the standard schedule in terms of stability of viral levels and therapeutic efficacy.

Based on these favourable results, anti-B cell therapy with rituximab represents a promising approach to the treatment of refractory MC. Nevertheless, problems remain with this treatment: (i) increased serum levels of HCV RNA; (ii) relapse of disease in up to 40% of cases; and (iii) no response in up to 20%. Controlled clinical trials are needed to establish definitively the efficacy of rituximab in this type of vasculitis.

ANCA-associated vasculitides. There are three main types of ANCA-associated vasculitis: Wegener's granulomatosis (WG), microscopic polyangiitis (MP) and Churg-Strauss syndrome (CSS). All three diseases are characterized by the presence in serum of autoantibodies that react with either neutrophil proteinase 3 (c-ANCA) or myeloperoxidase (p-ANCA). The level of circulating ANCAs appears to correlate with disease activity [97].

Rituximab treatment has been reported in 96 patients with relapsed or refractory ANCA-associated vasculitides, although most treated patients had WG (Table 3) [78, 98–106]. Rituximab administered with a lymphoma-like schedule induced a good response (close to 90%) in all studies, with the exception of one in which the overall response rate was 35% [102]. Vasculitis symptoms related to glomerulonephritis and small-vessel vasculitis quickly improved, whereas granulomatous manifestations regressed more slowly [102, 103]. Adverse events were rarely severe and were well controlled [99]. ANCA titres became negative after treatment in the majority of patients with a better outcome, although changes in ANCA level did not always correlate with disease activity. In some cases, disease remission occurred before the transitory drop in ANCA levels [78, 98], whereas relapse was not always preceded by a rise [101].

In two studies, the successful retreatment of responders after a relapse was reported [78, 101]. In a long-term prospective study (median follow-up, 33.5 months), rituximab was administered to 10

patients with ANCA-associated vasculitides according to a lymphoma-like schedule [101]; a relapse occurred in three patients in whom retreatment according to the same schedule was rapidly effective. Similar results were reported by others using a retreatment protocol of two 1-g doses of rituximab [78].

In conclusion, rituximab appears to have beneficial effects in patients with ANCA-associated vasculitides who fail to respond to or are intolerant to cyclophosphamide. However, the mechanisms of action remain unknown and the optimum dosage needs to be defined in large controlled trials. In this context, one ongoing randomized, double-blind study was registered in 2005 (NCT00104299.). This study has enroled 200 adults with WG and MP, and the first results are expected in March 2010.

Clinical trials in nonrheumatological autoimmune diseases

Rituximab has also been used experimentally in nonrheumatological autoimmune diseases, including haematological immune-mediated diseases (ITP and autoimmune haemolytic anaemia) and pemphigus.

Idiopathic thrombocytopenic purpura

In ITP, immunoglobulin auto-antibody-coated platelets are destroyed prematurely in the reticuloendothelial system. About 30% of adults with ITP do not respond to conventional therapy [107] and develop chronic refractory disease.

In a pilot study, a lymphoma-like dosage of rituximab was administered to 25 patients with chronic ITP [108]. A complete response (platelet count $>100 \times 10^3\ \mu L^{-1}$) was obtained in five cases, a partial response (platelets, $50–100 \times 10^3\ \mu L^{-1}$) in five cases, and a 'minor response' that did not require further treatment in another three cases. Thus an overall response rate of 52% was found, and a sustained response (longer than 6 months) was seen in 28% of patients. A significant increase in platelet count was observed in the second week of treatment. Young age and female gender were the only factors that predicted the response to treatment [108].

Other noncontrolled studies have confirmed the efficacy and safety of this drug in 380 patients (Table 4) [108–120]. The majority of patients received rituximab according to a lymphoma-like schedule. The overall response rate ranged between 40% and 90% and the beneficial effects of treatment lasted for more than 1 year in 40% of cases. The treatment was generally well tolerated, although one patient died from severe pneumonia which, however, was not directly attributable to rituximab due to the presence of comorbidities [112]. A systematic review of the literature published in 2007 identified 19 reports regarding the efficacy of rituximab for treatment of ITP (313 patients). Overall, 62.5% of patients were found to

Table 4 Clinical trials with rituximab in patients (pts) with idiopathic thrombocytopenic purpura (ITP) and autoimmune haemolytic anaemia (AHA)

Disease	No. of pts in each study [reference]	Total no. of pts (clinical subset, no.)	Rituximab dose; no. of infusions; timing.	Overall response (%)[a]	Adverse events (%)
ITP	25 [108]; 12 [109]; 12 [110]; 57 [111]; 35 [112]; 37 [113]; 18 [114]; 26 [115]; 60 [116]; 49 [117]; 14 [118]	345 (paediatric, 49)	375 mg m^{-2}; 4; weekly	40–90	Infections (1); death (0.3) serum sickness syndrome (0.3)
	7 [119]; 28 [120];	35	100 mg; 4; weekly	57–75	None
AHA	5 [124]; 5 [110]; 15 [125]; 2 [126]; 11 [127]; 27 [128]	65 (paediatric, 20)	375 mg $m^{-2;}$ 4; weekly	40–100	None

[a]Complete plus partial responses.

have a relevant clinical outcome lasting from 2 to 48 months [121].

Interesting data emerged from a retrospective study of patients with ITP who received four 100 mg weekly doses of rituximab, irrespective of body surface area or weight [119]. A complete and durable response occurred in four of seven patients (57%); this response rate is comparable to that achieved with conventional dosing. These results were confirmed in another study [120] that showed a complete response in 28 ITP patients following administration of the reduced dose of rituximab. Thus, in terms of B cell depletion and clinical response, a low dose of rituximab is as effective as the standard dose (lymphoma-like schedule), with the advantages of lower costs and reduced risks of adverse events. A long-term follow-up analysis of 18 ITP patients treated with rituximab showed an overall response rate of 56% (complete, 28%; partial, 28%) [114]. Median time to relapse was 21 months (95% CI, 15–93 months) in complete responders and 18 months (95% CI, 8–28 months) in partial responders.

Together, the following tentative conclusions can be drawn from these studies on the use of rituximab for ITP treatment: (i) the response of children with ITP to rituximab is comparable to that observed in adults [117]; (ii) rituximab represents a valid alternative to splenectomy, as both treatments provide long-term benefits [122]; (iii) rituximab can be effective in patients who have failed to respond to splenectomy [111]; and (iv) patients who do not respond to rituximab can still achieve a response after splenectomy [116]. Definitive conclusions will depend on the results of two ongoing randomized, double-blind, placebo-controlled clinical trials. One multicentre trial (NCT00344149, registered in 2006) will evaluate whether early rituximab treatment can reduce the need for splenectomy in patients who are unresponsive to corticosteroids; this study is currently recruiting and results are expected by the end of 2011. A pilot phase II trial (NCT00372892, registered in 2006) will assess the feasibility of adding rituximab to conventional therapy as first-line treatment for acute ITP in non-splenectomized adults; this study is currently recruiting and is expected to complete enrolment in 2009.

Autoimmune haemolytic anaemia

The reported efficacy of rituximab in other antibody-mediated autoimmune diseases provided the rationale for investigating its effects in autoimmune haemolytic anaemia (AHA). Although there are different forms of AHA, we will focus on idiopathic AHA this review as its clinical course is not influenced by other underlying disorders. About 10% of patients with AHA are resistant or unresponsive to conventional drugs [123].

Anti-CD20 treatment for AHA has been reported in 65 patients, including 20 children (Table 4) [110, 124–128]. In the largest case series involving 27 adults with refractory disease, the overall initial response rate was 93% (eight complete responses, 17 partial responses) and a relapse occurred in five responders at a median follow-up of 20.9 months [127]. In most studies, rituximab was administered according to a lymphoma-like schedule which was well tolerated by most patients; one severely immunocompromised patient contracted pneumocystis pneumonia [128]. Whether satisfactory results can also be achieved with low doses of rituximab, as in ITP, remains to be determined in controlled clinical trials. Fewer doses may also be an option, as suggested by a preliminary study that showed beneficial effects of one or two doses of rituximab (375 mg m^{-2}) in 11 of 12 patients with immune-mediated haematological disorders [129].

Pemphigus vulgaris and foliaceus

Pemphigus is an autoimmune disease mediated by pathogenic IgG4 auto-antibodies to desmoglein 1 (pemphigus vulgaris) or to cadherin-like glycoprotein (pemphigus foliaceus). This potentially fatal condition may become resistant or unresponsive to conventional therapies. To date, off-label use of rituximab has been reported in 54 patients with pemphigus.

In one study, rituximab was administered in two induction cycles (375 mg m^{-2} once weekly for 3 weeks and then 2 g kg^{-1} intravenous immune globulin (IVIG) on week 4, followed by 4 months of consolidation therapy (rituximab and IVIG at the

beginning of each month) [130]. This schedule induced rapid, long-lasting improvements of skin lesions, and serum IgG4 levels became undetectable in a mean period of 4.6 months; there were no reported adverse effects. Subsequently, beneficial effects of rituximab (using a lymphoma-like schedule) have been reported without the addition of IVIG [130–134]. Overall, rituximab induced a rapid resolution of skin lesions and clinical remission for several months; the rate of complete response ranged from 60% to 100% in these studies. However, two patients developed infections [132] and one died from septicaemia [134]. These events were not surprising in view of the wide extent of skin erosion that was present at the beginning of treatment. Recently, additional case reports have confirmed the efficacy of rituximab in this clinical setting [135–139], but controlled clinical trials are still needed.

Infections associated with rituximab infusion

Clinical trials have provided evidence that patients with autoimmune diseases treated with anti-CD20 mAbs are at risk of developing bacterial and viral infections. In the three largest controlled clinical trials in RA, infection was seen in 123 (16.5%) of the 745 rituximab-treated patients and in 74 (18.6%) of the 398 who received placebo; this was not a significant difference (Table 5). The infections were mainly of bacterial origin and most often involved the respiratory tract (sinusitis, nasopharyngitis, upper respiratory tract infections, bronchitis and pneumonia) [44–46]. A meta-analysis of these three trials demonstrated that the rate of severe infection was 2.3% in rituximab-treated patients and 1.5% in controls [140]. The authors concluded that rituximab treatment did not significantly increase the risk of infection. The results of another meta-analysis showed a 2.3% rate of serious infection in rituximab-treated RA patients [140], which is similar to the rate (3.7%) observed in RA patients treated with TNF-α inhibitors [141].

The risk of viral infection during rituximab treatment is unknown, although case reports have suggested an association. The increase in hepatitis C viral load following rituximab administration in MC patients has

Table 5 Rates of adverse events in patients (pts) with rheumatoid arthritis (RA) treated with rituximab (Rtx), in three major placebo-controlled double-blind randomized trials

	Clinical trial					
	Edwards *et al.* [44]		Emery *et al.* [45]		Cohen *et al.* [46]	
Adverse event	Rtx (pts no121)[a]	Placebo (pts no 40)[b]	Rtx (pts no 316)[c]	Placebo (pts no 149)[d]	Rtx (pts no 308)[d]	Placebo (pts no 209)[d]
Events (%)						
All events	79	80	84	70	85	88
RA exacerbation	12	40	15	30	21	42
Respiratory tract infection	11	15	13	13	23	23
Nausea or diarrhooea	15	3	13	14	13	10
Arthralgias	7	8	5	3	6	5
Hypertension	16	15	5	3	7	5
Urinary tract infection	NR[e]	NR	NR	NR	3	8
Fatigue	NR	NR	4	5	7	6
Dizziness	NR	NR	4	4	5	4
Fever	NR	NR	NR	NR	5	3

[a]Includes patients treated with Rtx alone, Rtx plus cyclophosphamide, and Rtx plus methotrexate (plus relevant placebos).
[b]Patients received methotrexate and placebo.
[c]Combines two groups of patients who received Rtx at different doses.
[d]All patients also received Mtx.
[e]Not reported.

been mentioned above. In addition, several cancer patients receiving rituximab and chemotherapy experienced reactivation of viral infections, including HBV [142], cytomegalovirus [143], varicella-zoster [144] and parvovirus B19 [145]. One patient with MC, who underwent renal transplantation, developed a disseminated herpes simplex infection [146], and two patients with WG experienced herpes zoster eruptions [100]. Of more concern, however, is the risk of progressive multifocal leukoencephalopathy (PML), which is a rare and usually lethal brain infection. Three patients died because of PML whilst receiving rituximab treatment for SLE (two cases) or RA, resulting in safety warnings by the US FDA in 2006 and 2008, respectively (http://www.fda.gov/Safety/MedWatch/SafetyInformation/SafetyAlertsforHumanMedicalProducts/default.htm). PML is caused by the JC virus, a polyomavirus present in latent form in many healthy individuals without clinical sign of infection [147]. The virus is known to reactivate in immune-depressed patients (e.g. transplant recipients and those with HIV infection) and to destroy oligodendrocytes and astrocytes, leading to white matter demyelinization. However, a recent report described 57 HIV-negative patients who developed PML following rituximab treatment for lymphoproliferative disorders (52 cases) and autoimmune diseases (five cases) with a fatality rate of 90% [148].

A clear association between PML and rituximab cannot be established at present, because: (i) PML has been reported to develop in patients with rheumatic diseases (mainly SLE) receiving conventional (nonbiological) immunosuppressive drugs [149]; and (ii) B cell depletion should have no effect on the activation of this virus [150]. Even so, as suggested by Calabrese and Molloy [151], continued vigilance is needed when administering biological immunosuppressants to patients with autoimmune diseases. Obtaining informed consent from patients regarding this risk should also be mandatory.

The use of anti-viral prophylaxis may also be considered in rituximab-treated patients who have experienced at least one episode of herpes zoster infection. Based on experience in cancer patients receiving chemotherapy [152], low doses of acyclovir (400–800 mg day^{-1}) during rituximab therapy might reduce the risk of a severe reactivation of this viral infection and thus also avoid discontinuation of the biological therapy. The efficacy or safety of anti-viral prophylaxis during rituximab treatment has not been assessed in a clinical trial. One potential risk of such co-therapy is a higher probability of viral mutation, leading to the appearance of strains resistant to anti-viral therapy [153].

Conclusions

The effectiveness of rituximab in RA, which has been demonstrated in three large clinical trials, has opened the way to explore its efficacy in other autoimmune diseases in which B cells are thought to play a pathogenetic role. The results of many noncontrolled clinical trials and case studies have suggested that rituximab is effective in SS, SLE, MC, WG, ITP, AHA and pemphigus, whilst questionable results have been obtained in DM/PM. Unusually high rates of infection in pemphigus and of hypersensitivity reactions in SS are, nonetheless, aspects of concern. Despite the encouraging results that emerged from preliminary studies for many of these autoimmune diseases, evidence from large controlled clinical trials is still required; although trials are ongoing for several of these diseases, none has been registered (with the NIH) to investigate the use of rituximab in the treatment of MC, AHA or pemphigus.

Progressive multifocal leukoencephalopathy, which recently emerged as a potential, lethal adverse event in rituximab-treated patients, represents a safety caveat to this therapeutic approach. PML occurs very rarely and a causal association with rituximab infusion has not been demonstrated. Even so, efforts are being made to identify patients at higher risk of the disease and to develop anti-viral therapy to prevent or cure it. Despite these concerns, inclusion of CD20-depleting immunotherapy in the medical armamentarium for autoimmune diseases offers novel possibilities of caring for patients refractory to traditional treatments. As new humanized and fully human mAbs become available, we can expect a further improvement in the efficacy and safety of biological immunotherapy.

Conflict of interest statement

The authors declare that they have no competing interests.

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