Rituximab – The First Twenty Years

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Introduction:

It was, as the Beatles so nearly put it, approximately twenty years ago today the first attempt to treat patients with active systemic lupus erythematosus with B-cell depletion was undertaken at the Centre for Rheumatology, University College Hospital. We reported the first 6 patients that we treated with a six-month follow-up in 2002[1] and numerous open-label studies [e.g. 2-8] and some large-scale clinical trials have been published since [e.g. 9-11].

Rituximab is a monoclonal antibody directed against the B-cell specific antigen CD20 which acts as an efficient depleter of B-cells in the peripheral blood [12]. Initially used in the treatment of non-Hodgkin's lymphoma, it was formally approved for its use in this condition by the Federal Drug Administration in 1997. Our colleagues Professor Joe Edwards and Geraldine Cambridge, based at University College London, became convinced that it would be a useful approach in the treatment of rheumatoid arthritis and the first patients with this condition were treated in 1999 [13]; impressive ACR 50 and 70 responses were noted, the drug met its primary endpoint in a major clinical trial [14] and is now widely used in the treatment of this disease.

The Use of Medicines Committee at University College Hospital granted us permission to treat lupus patients with B-cell depletion – initially for those who had failed conventional immunosuppressive drugs. This provided us with the necessary "ticket to ride" and in this review, we will highlight the history of rituximab usage in lupus in the past two decades, some more recent advances and discuss what the future might be for B-cell depletion in SLE.

Further history:

Lupus studies, whether clinical trials or open-label follow-up studies, invariably divide into those focusing on lupus nephritis and others looking at non-renal lupus. The therapeutic use of rituximab in non-renal lupus was reviewed by Cassells et al [7] in patients known to be resistant to steroids and immunosuppressive drugs. They reported clinical benefit in 91% of patients using either the

lymphoma regime (375mg per m^2) of rituximab given weekly for four weeks or the original dosing regime [1] i.e. 1g given two weeks apart. They reported a slightly higher rate of response in patients given the four-weekly regime (94% versus 3%; p = 0.048). In a more recent single-centre study, we have reviewed the first 165 patients with lupus treated with rituximab [15] the clear majority – (more than 50% not having nephritis) were treated by the two x 1g infusion regime. Using the British Isles Lupus Assessment Group (BILAG) Disease Activity Assessment with the classic A-E scores converted into numbers using the A = 12, B = 5, C = 1, D/E = 0 scoring [16] we reported that 73% of these patients showed a reduction in their BILAG score by 5 points or more, sustained for up to 6 months post-treatment. The study aimed to see whether particular clinical features, or standard biomarkers, might identify patients less likely to respond, but we were unable to achieve this. tThe reasons why approximately 10-15% of patients with lupus do not respond remain obscure, though part of the explanation depends upon the variability in the capacity of the approach to achieve complete B-cell depletion [17]. This is discussed in more detail below.

In a series of studies from our own centre we have shown that repeated cycles of rituximab are safe and effective for active refractory lupus [18]. Rituximab reduces levels of antibodies to DNA, nucleosomes and cardiolipin, but does not change the levels of antibodies to Ro, Sm, RNP and La [19-21]. The more complete and longer the duration of B-cell depletion the longer the clinical benefit [22]. Used at the time of diagnosis, or shortly afterwards rituximab is both effective and reduces the cumulative dose of corticosteroids substantially. [23] Its capacity to improve skin rashes depends upon the type of rash involved. [24] One particular cytokine, B-cell activating factor BAFF, appears to be particularly influential in determining the time to flare post-rituximab in lupus patients linked to the return of the CD20 positive B-cells [25].

Vital and colleagues [22] using a highly sensitive flow cytometric method which can enumerate B-cells at levels 50-100 lower than the conventional techniques. They reported that an incomplete B-cell depletion six weeks after treatment was linked to lower clinical responses at 26 weeks. Relapses in the main do not become obvious until the repopulation of memory B-cells and plasma blasts in particular has occurred. We have one notable patient treated in 2001 with two 1g rituximab infusions who remains B-cell depleted (by conventional assays) and in effect in clinical remission, but who still has raised levels of antibodies to double-stranded DNA, which we assume must be coming from B-cells/plasma cells "hidden" within the organs of the body notably the liver and spleen.

Renal disease:

An array of studies [e.g. 4,7,8, 26- 29] looking at lupus nephritis have been published ([e.g. 4,7,8, 26- 29]). It is worth nothing that the precise definitions of partial or complete remission differ a little between the individual studies, so that comparisons between them must be made with some caution. Nevertheless it is obvious from these studies, that many patients with established nephritis who failed standard drug regimes utilizing steroids and cyclophosphamide or mycophenolate, have been getting better . These data have been sufficient to allow working parties for both the American College of Rheumatology [30] and the European League Against Rheumatism [31] to recommend the use of rituximab in patients with lupus nephritis.

More recent developments:

Excitingly, Professor Lightstone and colleagues at the Hammersmith Hospital in London [32] showed that of 50 patients with biopsy-proven lupus nephritis given rituximab shortly after diagnosis when treated with 1g x 2 followed by mycophenolate and hydroxychloroquine, approximately 90% achieve complete or partial remission without requirement for oral steroids. Indeed, during a two-year follow-up period only 2 of 50 patients had flares requiring oral steroids. This approach has been supported by studies done at University College Hospital [33] in which 16 patients reviewed for periods of 1-6.5 years and given rituximab followed by azathioprine and hydroxychloroquine at or close to the time of diagnosis, were matched with 3 patients (for sex, age, type and duration of symptom), given conventional therapy with oral steroids. The B-cell depleted patients had slightly fewer flares, but even more impressive was the substantial reduction in oral steroids required during follow-up. Several of these 16 patients received only 250mg (which was given together with the rituximab) of methylprednisolone throughout the whole follow-up period, whereas the controls invariably received in excess of 3gm of steroids. Most unfortunately a clinical trial, RITUXILUP, which aimed to confirm these observational studies was beset by problems including the premature loss of funding for the drug, and challenges with recruiting sufficient numbers of patients so that formal clinical trial confirmation of this approach has not been achieved. Nevertheless there remains the remains a tantalising prospect of lupus patients getting better with a substantial saving in corticosteroid use.

Clinical trials:

Two randomised controls trials one in lupus nephritis (LUNAR) [10] and one in extra-renal lupus (EXPLORER) [9] compared patients treated with standard of care given either placebo or Rituximab. The primary endpoints were not met in these trials although detailed post-hoc analyses do reveal a number of very encouraging trends (see Table 1). Nevertheless, it clearly has been of importance to try to determine, if we can work it out, why the primary endpoints were not met. Possible explanations include the "over-indulgence" in the use of concomitant corticosteroids allowed in these trials together with high background immunosuppressive regimes, the relatively slow nature of the steroid tapering advocated, whether the follow-up periods were long enough, the powering of the sample size and, perhaps the inability of the disease activity instruments to capture response adequately.

Side-effects of B cell depletion therapy based on rituximab:

Treatment with rituximab in patients with SLE can be associated with side effects, including infusion reactions on retreatment in patients previously exposed to the drug [34]. These infusion reactions can preclude re-exposure to the drug and in the authors' experience, tend to reoccur even when desensitisation protocols are used. At least a significant part of these infusion reactions are thought to be due to the development of anti-drug antibodies [35,36]. The occurrence of serum sickness type of reactions is considered to be a contraindication to re-exposure to rituximab.

Rituximab has been associated with both early- (within one month) and late-onset neutropaenia (up to one year, commonly around 4 to 6 months) which is frequently self-limited [37,38,39]. When associated with symptoms of infection there is usually a good response to treatment with filgrastim. The exact mechanism for the late neutropaenia is not fully understood and central (bone marrow based) mechanisms have been suggested.

Repeated cycles of treatment with rituximab are associated with an increased risk of hypogammaglobulinaemia (40-3). This effect most commonly involves IgM followed by IgG and rarely IgA (41,43). Although, so far, no clinically significant associations have been reported with isolated low IgM, the occurrence of low IgG has been associated with an increased risk of serious or repeated infections in particular sino-respiratory infections.

Rituximab is usually well tolerated. Nevertheless, as with any immunosuppressive treatment, rituximab can increase the risk of infections, including rare opportunistic infections (40,44). It is often difficult to distinguish between the risks associated with treatment with rituximab from risks associated with the disease itself, or with concomitant or previous therapies, particularly in patients without hypogammaglobulinaemia (40). Recurrent herpes zoster infections are thought to be the most common opportunistic infections reported. Very rare cases of progressive multifocal leukoencephalopathy (PML) have been reported in patients treated with rituximab but there does not seem to be a particular predilection for it cause this sinister disease, more than any other kind of immunosuppression (45).

Optimisation strategies of B cell targeting in SLE:

Data including that from the initial open label studies have suggested that the degree and duration of B cell depletion achieved with standard doses of rituximab in patients with SLE is variable and less predictable than, for example, the one achieved with the same doses in patients with rheumatoid arthritis (1, 46-48). Data also suggests that insufficient depletion can be associated with lack of or less good response to treatment (12, 49). In addition, the increase in serum BAFF levels associated with B cell depletion following rituximab treatment has been put forward as a possible mechanism for increased likelihood of survival of autoreactive B cell clones (19,24). This knowledge has led to the development of treatment strategies to improve the effectiveness of B cell depletion treatment in SLE including the use of newer generation anti-CD20 monoclonal antibodies and of combination therapy with belimumab, a monoclonal antibody to soluble BAFF.

Improving B cell depletion with other anti-CD20 monoclonal antibodies:

In patients who have previously responded to rituximab and have either experienced infusion reactions that preclude re-exposure to the drug or did not deplete as well as expected, treatment with other anti-CD20 monoclonal antibodies including ofatumumab and ocrelizumab has been reported as usually well tolerated and effective (50-2). More recently, the use of newer generation monoclonal anti-CD20 antibodies, with some differences in the interaction with the CD20 target and with modifications that lead to a stronger interaction with Fc receptors on effector cells has been explored in vitro in samples of patients with SLE (53, 54). One such antibody, obinutuzumab has been reported as effective in treating lupus nephritis. A full peer-reviewed report is awaited (55).

Combination therapy with belimumab:

BAFF is an important cytokine involved in B cell survival and B cell activation. A possible role for BAFF in supporting the survival and function of autoreactive B cell clones has been proposed from SLE based and animal model and in vitro and clinical study data (56). The idea of combining B cell depletion with rituximab with blocking BAFF to target B cell survival/activation aiming for synergy or an additive effect between the two approaches has been suggested by several authors and explored in animal models (57, 58). Belimumab, a monoclonal antibody directed to soluble BAFF has been proven effective in improving disease control in patients with SLE (59) and has been approved for its treatment. A small number of clinical cases where rituximab and belimumab were used in sequence and a combination phase 2, single-arm, open-label proof of concept study have been published (60-62). There are ongoing randomised clinical trials with slightly different strategies and their results are awaited with interest. Depending on the trial, the main objective is to increase the likelihood of depletion of pathogenic B cell clones and/or to decrease the likelihood of resurgence of pathogenic autoreactive B cell clones following the initial depletion (61,62).

B cell depletion – future prospects:

Despite the fact that the two RCT of rituximab for the treatment of refractory active lupus nephritis or non-renal disease in SLE patients did not show efficacy of rituximab added to standard treatment, B cell depletion therapy based on rituximab continues to be widely used in clinical practice and is included as a treatment option in several guidelines. Particularly in refractory lupus nephritis and in patients with haematological manifestations such as autoimmune thrombocytopaenia and haemolytic anaemia there is a place for rituximab. Its potential use as a first line agent remains a tantalizing possibility.

Whether a patient with active SLE responds or not to rituximab, how good the response is and how long does it last is likely to depend on multiple factors. It is important to acknowledge that the extent and duration of the B cell depletion achieved with standard rituximab doses will vary from patient to patient. Rituximab depletes B cells by recruiting the patient's immune system to cause B cell death mainly through antibody-dependent cellular cytotoxicity and antibody-dependent cellular phagocytosis. Complement activation is also thought to be important with a smaller possible contribution from direct cell death. Individual differences in rituximab-target cell interaction and in

engagement and effectiveness of the different immune effector mechanisms are likely to be clinically relevant. In addition, some clinical manifestations are likely to be more dependent than others on ongoing formation of new plasma cells producing pathogenic autoantibodies and therefore more likely to respond to B cell depletion and the interruption of this process.

These factors may also vary over time and it is possible that the likelihood of achieving good depletion and of responding to B cell depletion therapy can be different for different patients and also in individual patients at different times of their disease course. While in rheumatoid arthritis, if patients respond to B cell depletion therapy with rituximab, their disease is frequently controlled with repeated cycles of treatment (unless patients either stop responding to treatment or develop side effects that justify discontinuation), its use in a disease such as SLE is often different. While in the case of lupus nephritis it makes sense to opt for a prolonged course of treatment and use a second course of treatment to maintain B cell depletion and improve/sustain control of disease activity, in patients with acute onset of autoimmune thrombocytopaenia or haemolytic anaemia one rituximab course may lead to a long-lasting response and repeated courses of treatment may not be needed. When deciding to use rituximab to control disease activity and/or induce remission it is important to think of strategies of how to maintain disease control if this is expected to be needed. In some patients this may involve repeated cycles of rituximab.

In conclusion, B cell depletion therapy based on rituximab was first used to treat patients with SLE twenty years ago and although a long and winding road including two negative clinical trials it has become a useful tool in the treatment of patients with active, refractory disease. Use of alternative anti-CD20 monoclonal antibodies in patients who have previously respond to treatment and have had infusion reactions that preclude retreatment or have not depleted well should be considered in SLE patients with the main limitation being availability of the drug (ofatumumab) or cost (ocrelizumab and obinutuzumab). Current optimisation treatment strategies are being tested in clinical trials. These include using newer generation anti-CD20 monoclonal antibodies such as obinutuzumab which are likely to improve the degree, extent and duration of B cell depletion and combination therapies with belimumab, to either improve depletion particularly in solid tissues and/or delaying/preventing the repopulation of pathogenic B cell clones by concomitant targeting of BAFF. We believe there really is a place for the use of rituximab [and the newer B cell depleting agents] in the management of lupus throughout the duration of the disease. We hope that it won't be long before more 'official recognition' to this approach is granted.

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TABLE 1

EXPLORATORY ENDPOINTS – 78 WEEKS	Placebo	Rituximab	p
At least 50% reduction in proteinuria	54.2%	70.8%	0.04
CR or PR proteinuria	56.9%	73.6%	0.04
Black patients overall response 52 weeks	45%	70%	ns
Black patients overall response 78 weeks	35%	60%	ns
Started CyP prior to week 52	11%	0%	0.006
Started CyP prior to weeks 78	15.3%	2.8%	0.02
Average daily dose pred week 16-52	12.8$\pm$6.5	10.9$\pm$ 4.1	0.05

Data taken from references 9 and 10.

CR = complete response

PR = partial response

CyP = Cyclophosphamide

Pred = Prednisolone