TRANSPLANT INTERNATIONAL

Transplant International ISSN 0934-0874

REVIEW

Rituximab in renal transplantation

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Keywords
monoclonal antibodies, CD20 antigens, B-lymphocytes, graft rejection, immunosuppression, kidney transplantation.

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Conflicts of interest
NM is Chief Investigator and ANRB is a Co-Investigator of the ongoing randomized controlled clinical trial, ReMIND (RituxiMab INDuction in renal transplantation, NCT01095172).

Received: 11 October 2012
Revision requested: 9 November 2012
Accepted: 7 January 2013
Published online: 18 February 2013

doi:10.1111/tri.12072

Summary

Rituximab is a chimeric anti-CD20 monoclonal antibody that leads to B cell depletion. It is not licensed for use in renal transplantation but is in widespread use in ABO blood group incompatible transplantation. It is an effective treatment for post-transplant lymphoproliferative disorder, and is also used in both HLA antibody incompatible renal transplantation and the treatment of acute rejection. Recent evidence suggests rituximab may prevent the development of chronic antibody mediated rejection. The mechanisms underlying its effects are likely to relate both to long-term effects on plasma cell development and to the impact on B cell modulation of T cell responses. Rituximab (in multiple doses or in combination with other monoclonal antibodies and/or other immunosuppressants) may lead to an increase in infectious complications, although the evidence is not clear. Rarely, the drug can cause a cytokine release syndrome, thrombocytopenia and neutropenia. It has been related to an increased risk of progressive multifocal leucoencephalopathy and, recently, deaths from cardiovascular causes. Trials examining the effects of rituximab in induction therapy for compatible renal transplantation and the treatment of chronic antibody mediated rejection are ongoing. These trials should aid greater understanding of the role of B-cells in the alloresponse to renal transplantation.

Introduction

Rituximab is a chimeric anti-CD20 monoclonal antibody licensed for use in non-Hodgkin's lymphoma (NHL), chronic lymphocytic leukaemia (CLL) and rheumatoid arthritis (RA) [1]. The CD20 antigen is a transmembrane nonglycosylated phosphoprotein, expressed on immature and mature B cells. It is associated with transmembrane calcium conductance and the regulation of cell proliferation and differentiation [2].

The rituximab Fab domain binds to the CD20 antigen; the Fc domain can recruit immune effector functions [1]. Once rituximab has bound to the CD20 antigen, it affects B cells in at least three ways [3]:

1. Activation of the complement cascade, leading to complement-mediated cytotoxicity
2. Macrophage recognition, leading to phagocytosis and antibody-dependent cell-mediated cytotoxicity (ADCC)
3. Natural killer cell interaction, also leading to ADCC.

Rituximab causes a reduction in B cells in the peripheral blood within 1–3 days of administration, and complete B cell depletion in the majority of patients within 1–6 weeks [4]. It does not have a direct effect on plasma cells (which do not express the CD20 antigen).

Clinical use of rituximab in renal transplantation

Rituximab is not licensed for use in renal transplantation, but is used 'off-label' in a variety of situations.

Induction/desensitization in antibody incompatible transplantation

ABO blood group incompatible transplantation

In the early days of ABO blood group incompatible (ABOi) renal transplantation, splenectomy was considered mandatory. Rituximab was first used in the context of ABOi renal transplantation (in combination with double filtration plasmapheresis and splenectomy) in 2002 [5]. Some centres have continued to use both rituximab and splenectomy [6] [in patients with high anti-A/B antibody titres and/or the presence of HLA donor specific antibody (DSA)].

The first description of rituximab to replace splenectomy as desensitization in ABOi renal transplantation came from Stockholm in 2003 [7]. Its use in this context has now become widespread ([8–23], see Table 1). Rituximab is used commonly in Japan, and the Stockholm approach has been widely adopted throughout Europe, with some modifications in pretransplant [24] and post-transplant antibody removal [25]. At Guy's Hospital a protocol of minimizing desensitization depending on the initial antibody titres is used [26].

The allograft survival and patient survival rates for published reports of ABOi renal transplantation (over varying time-frames) using rituximab between 2004 and 2012 are summarized in Table 1. For comparison, the 1-year results for living donor renal transplants in the United Kingdom between 2007 and 2010 are allograft survival of 96% and patient survival of 99% [27].

Generally, the accepted dosing strategy has been 375 mg/m^2; lower doses have been used with no differences in clinical outcomes [20,22]. While most centres have maintained the use of rituximab in place of splenectomy, others have omitted it entirely from their desensitization programmes [28,29] or replaced it with alemtuzumab [30].

HLA antibody incompatible transplantation

Table 2 summarizes published results of outcomes from centres [31–38] that have used rituximab as part of their desensitization strategy for HLA antibody incompatible (HLAi) renal transplantation.

There are differences in the way that rituximab has been used in HLAi renal transplantation as opposed to ABOi transplantation, both in the timing of administration (it is often given at the time of transplantation or even post-transplant) and in the concomitant desensitization strategies used (it is more likely to be used in combination with other treatments).

Mechanisms of action

In ABOi renal transplantation, the main risk arises from high anti-A or anti-B antibodies. Antibody removal strategies are effective in reducing the antibody to acceptable levels at the time of transplant. Evidence is beginning to emerge that rituximab prevents antibody from increasing in the medium to long-term, and reduces levels of chronic antibody mediated rejection (CAMR, see below). In ABOi renal transplantation therefore, rituximab's effects seem to arise from a direct effect on the B cell/plasma cell development pathway.

In the immunologically more complex HLAi renal transplantation, the effects of rituximab are likely to arise from impairing B cell regulation of T cells (such as CD8+ recall responses [39] and CD4+ activation [40]). It has recently been suggested that rituximab may also cause inactivation of T cells directly [41]. As it takes 1–6 weeks for B cell depletion to be complete, there is a rationale for suggesting the earlier administration of rituximab in HLAi transplantation.

Rituximab as a treatment for acute renal allograft rejection

Almost all the reports of the use of rituximab as a treatment for acute renal allograft rejection (in addition to a variety of other treatments including plasmapheresis, steroids, OKT3, IVIG, alemtuzumab and ATG) have been purely descriptive, either single-case reports [42–47] or case series [48–55] (see Table 3). There has been only one randomized controlled trial: Zarkhin *et al.* [56] randomized 20 paediatric patients with biopsy proven acute rejection (BPAR) and a finding of B cell infiltrates in their renal allograft to receive either four doses of rituximab or no additional treatment. (All patients received either steroids and/or ATG.) Patients in the rituximab arm had worse rejection before treatment than patients in the control arm. Six months post-transplant, rituximab-treated patients had statistically significantly lower acute rejection scores than patients in the control arm, and had better creatinine clearance after treatment. This is a small study, but in combination with the case series, does offer evidence that rituximab may have some effect in the treatment of acute AMR as part of a broad pharmacological approach, either in combination with other treatments or as a treatment of last resort after other therapies have proved ineffective.

Rituximab leads to a reduction in B cells within allografts when given as induction therapy [4], and also when given as treatment for rejection [57]. An association between improvement in allograft function and reduction in B cells after rituximab treatment for rejection has been described [42]. There is emerging evidence that B cells play a central role in the formation of Tertiary Lymphoid Organs (TLOs) and the modulation of chronic rejection [58]. This all suggests that rituximab has local effects in addition to the generalized effects described above.

Table 1. Results from adult ABOi renal transplant programmes using rituximab.

Authors	Year	Centre	Number of patients	Dosing of rituximab	Follow-up	Allograft survival	Patient survival	Acute AMR	TCMR
Sonnenday *et al.* [8]	2004	Johns Hopkins, USA	6	375 mg/m^2 1 day prior to transplantation	12 months (median)	100%	100%	0%	17%
Gloor *et al.* [9]	2005	Mayo Clinic, USA	11	Single dose within 48 h prior to transplantation	399 days (mean)	82%	91%	18%	Not stated
Saito *et al.* [10]	2006	Niigata, Japan	6	375 mg/m^2 on days −14 and −1	Not stated	83%	100%	0%	33%
Chikaraishi *et al.* [11]	2008	Kawasaki, Japan	8	100 mg on days −8 and −1	1–18 months	100%	100%	25%	0%
Galliford *et al.* [12]	2008	Imperial College, UK	10	1 g on days −14 and day of transplant	18.6 months (median)	100%	100%	30%	20% (in conjunction with AMR episodes)
Haidinger *et al.* [13]	2009	Vienna, Austria	4	375 mg/m^2 4 weeks prior to transplantation	4–18 months	100%	100%	0%	0%*
Oettl *et al.* [14]	2009	Basel, Switzerland	10	375 mg/m^2 4 weeks prior to transplantation	489 days (median)	100%	100%	Not clearly stated	Not clearly stated
Tanabe *et al.* [15]	2009	Toda, Japan	24	500 mg on day −7	3 years	96%	100%	8% within 6 months	8% within 6 months
Schousboe *et al.* [16]	2010	Odense, Denmark	11	375 mg/m^2 4 weeks prior to transplantation	8 months (median)	100% (death censored)	91%	9%	9%
Wilpert *et al.* [17]	2010	Freiburg, Germany	40	375 mg/m^2 4 weeks prior to transplantation	39 months (median)	100% (death censored)	98%	5%	23%
Habicht *et al.* [18]	2011	Hannover, Germany	21	375 mg/m^2 4 weeks prior to transplantation	16.9 months (mean)	100% (death censored)	95%	5%	14%
Genberg *et al.* [19]	2011	Stockholm, Sweden	36	375 mg/m^2 4 weeks prior to transplantation	50.0 months (median)	89%	94%	6%	11%
Shirakawa *et al.* [20]	2011	Tokyo, Japan	24	500 mg between days −7 and −5	55.0 months (mean)	96%	100%	4%	16%
Shirakawa *et al.* [20]	2011	Tokyo, Japan	50	200 mg between days −7 and −5	21.8 months (mean)	98%	100%	2%	6%
Fuchinoue *et al.* [21]	2011	Tokyo, Japan	50	Variable (100 mg, 200 mg or 500–1000 mg, between 1–3 doses)	36.4 months (median) for all patients (not just ABOi with rituximab)	100%	100%	4%	4%

Table 1. continued

Authors	Year	Centre	Number of patients	Dosing of rituximab	Follow-up	Allograft survival	Patient survival	Acute AMR	TCMR
Chung *et al.* [22]	2011	Seoul, South Korea	14	100 mg/m^2 or 375 mg/m^2 4 weeks prior to transplantation	Not stated	Not stated	Not stated	0%	21%
Yoshimura *et al.* [23]	2012	Kyoto, Japan	24	200 mg on days 14 and 7 before transplant	1 year	100%	100%	0% at 3 months	13% at 3 months
Morath *et al.* [24]	2012	Heidelberg, Germany	17	375 mg/m^2 4 weeks prior to transplantation	18 months	100% (death censored)	94%	0%	0%

ABOi, ABO blood group incompatible; AMR, antibody mediated rejection; TCMR, T cell mediated rejection.
*although one patient (25%) treated with steroids for graft dysfunction in the presence of cellular infiltrates and C4d deposition in the absence of morphological features of cellular rejection.

Rituximab and CAMR

Treatment of CAMR

There have been fewer studies examining the use of rituximab in the treatment of CAMR as opposed to acute rejection. These studies have shown improvement following the administration of rituximab in combination with other therapies in some patients.

Billing *et al.* [59] treated six paediatric patients who developed CAMR with IVIG and a single dose of rituximab (375 mg/m^2). GFR improved or stabilized in four of the six patients after treatment, and continued to deteriorate in two. Fehr *et al.* [60] report on four patients diagnosed with CAMR treated with steroids and rituximab (375 mg/m^2), three of whom also received IVIG. Six months after treatment with rituximab, the GFR was significantly improved from that before rituximab ($P = 0.009$).

Rituximab has also been used in the treatment of transplant glomerulopathy [61]: of 14 renal transplant recipients with deteriorating allograft function, seven lost their allografts and seven had stabilization of renal function.

These reports are not conclusive and there is an ongoing randomized controlled trial which should provide a definitive answer. The RituxiCAN-C4 trial (NCT00476164) is designed to 'determine whether anti-CD20 therapy can stabilize or improve renal function and/or proteinuria in patients with C4d+ chronic (humoral) rejection in whom standard therapeutic approaches have failed' [62].

Prevention of CAMR

There have been two published reports that suggest B cell depletion may play a role in preventing the onset of CAMR: Loupy *et al.* [35] found that patients with DSA and a negative cytotoxic crossmatch who received rituximab and plasmapheresis in addition to IVIG and ATG had lower rates of CAMR at 1 year post-transplant than those who did not receive rituximab and plasmapheresis (13.3% compared with 41.3%, $P = 0.03$). Similarly, Kohei *et al.* [63] identified that ABOi renal transplant recipients had a statistically significant lower rate of CAMR at 2 years post-transplant than patients who received a compatible transplant (22.9%), and that those ABOi recipients who received rituximab had a lower rate than those who underwent splenectomy (3.5% and 8.8% respectively, although this difference was not statistically significant).

These are early findings. It is possible that rituximab does lead to long-term effects on the B cell repertoire, so leading to a reduction in CAMR, but the mechanisms underlying this effect have not been identified as yet.

Rituximab as induction therapy in compatible renal transplantation

The success arising from the use of rituximab, particularly in ABOi renal transplantation, suggests that B cell

Table 2. Results from HLAi renal transplant programmes using rituximab.

Authors	Year	Centre	Number of patients	Definition of HLA incompatibility used	Dosing of Rituximab	Follow-up	Allograft survival	Patient survival	AMR	TCMR
Gloor *et al.* [31]	2003	Mayo Clinic, USA	14	CDC +ve	375 mg/m^2 on day 4 post-transplant	448 days (mean)	79%	86%	14% with rise in creatinine; further 29% diagnosed on protocol biopsy and treated	0%
Munoz *et al.* [32]	2008	Quezon City, Philippines	7	PRA >30%, >3 pregnancies or history of +ve T cell crossmatch	375 mg/m^2 on day 1 prior to transplant (one patient received two doses, 2 weeks and 1 day prior to transplant)	3 months (mean)	100%	100%	29%	Not stated
Magee *et al.* [33]	2008	Brigham and Women's Hospital, USA	28	CDC +ve	375 mg/m^2 on day −1 or day 1. Additional dose 3–4 weeks prior to transplant in those deemed high-risk	Not stated	89%	93%	39%	42%
Yin *et al.* [34]	2009	Beijing, China	7	PRA >30%	375 mg/m^2 on day of transplant	Not stated	100%	100%	0%	43%
Loupy *et al.* [35]	2010	Necker Hospital, France	18	Preformed DSA	375 mg/m^2 on day 4 post-transplant (repeated depending on CD19+ cell count)	19.5 months (mean)	89%	94%	17%	Not stated
Vo *et al.* [36]	2010	Cedars-Sinai, USA	76	PRA >30% and +ve T cell FCXM or DSA	1 g on day 15 pretransplant	18.8 months (mean)	84%	95%	29%	8%
Yin *et al.* [37]	2011	Beijing, China	7	PRA >30%	375 mg/m^2 on day −1	7–12 months	100%	100%	0%	29%
Morath *et al.* [38]	2012	Heidelberg, Germany	10	9 CDC +ve, 1 DSA	375 mg/m^2 on day −1	19 months (median)	90%	100%	30%	20%

AMR, antibody mediated rejection; DSA, donor specific antibody; HLAi, HLA antibody incompatible; TCMR, T cell mediated rejection; CDC, complement dependent cytotoxic crossmatch; FCXM, flow cytometric crossmatch; PRA, panel reactive antibody.

(i) Reported definitions of HLA antibody incompatibility vary between Centres. (ii) Where Centres have used rituximab for only some patients, results have been included only where differentiation has been made between those who did and did not receive rituximab.

Table 3. Rituximab as treatment for acute rejection

Authors	Year	Centre	Number of patients	Dosing of rituximab	Follow-up	Allograft survival	Patient survival
Case series							
Becker *et al.* [48]	2004	Wisconsin, USA	27 patients with BPAR, either AMR or resistant to initial treatment	375 mg/m^2, one dose	605 days (mean)	85% (death censored)	78%
Faguer *et al.* [49]	2007	Toulouse, France	8 patients with AMR	375 mg/m^2, four doses	10 months (median)	75%	100%
Tanriover *et al.* [50]	2008	Dallas, USA	7 patients with AMR	375 mg/m^2, one dose	2 years	58%	100%
Mulley *et al.* [51]	2009	Clayton, Australia	7 patients with AMR	500 mg, one dose	20 months (mean)	100%	100%
Gomes *et al.* [52]	2009	Porto, Portugal	4 patients with refractory AMR	375 mg/m^2, one dose	1–18 months	100%	100%
Rodriguez- Ferrero *et al.* [53]	2010	Madrid, Spain	2 patients with AMR	375 mg/m^2, one dose	Not stated	50%	100%
Kaposztas *et al.* [54]	2009	Houston, USA	26 patients with AMR	375 mg/m^2, multiple doses	2 years	90%	100%
Lefaucher *et al.* [55]	2009	Paris, France	12 patients with AMR	375 mg/m^2, two doses	36 months	92%	100%
Randomized controlled trial							
Zarkhin *et al.* [56]	2008	Stanford, USA	10 paediatric patients with BPAR	375 mg/m^2, four doses	1 year	80%	100%

AMR, antibody mediated rejection.

depletion may also be of value as induction in antibody compatible renal transplantation, but there are few randomized controlled trials in this area. Tyden *et al.* [64] performed the first trial examining the use of rituximab in antibody-compatible renal transplantation. One hundred and forty adult renal transplant recipients were randomized to receive either a single dose (375 mg/m^2) of rituximab or a placebo within 24 h of transplantation. The primary end-point was composite, defined as treatment failure, including BPAR, graft loss or death within 6 months of transplantation – the trial was powered to identify a reduction in this composite end-point from 18% to 3%. No statistical difference was found in the number of treatment failures between the two groups. Although there were more BPAR episodes in the control group than the rituximab group (17.6% vs. 11.6%) this difference was not statistically significant. The authors suggest that the reduction in composite end-point was less than that included in the power calculation because of a difference in the timing of rituximab administration. In this trial rituximab was given immediately prior to transplant to incorporate deceased donor as well as living donor renal transplants, whereas in the same centre's ABOi transplant programme rituximab is given 30 days prior to transplant [19].

In contrast, Clatworthy *et al.* [65] halted a randomized controlled trial, in which they planned to recruit 120 patients, after the recruitment of only 13 patients when they identified a higher rate of T cell mediated rejection in the rituximab arm. Patients in the control arm received two doses of daclizumab; patients in the rituximab arm received two doses of methylprednisolone and rituximab. All patients received maintenance immunosuppression with tacrolimus and mycophenolate mofetil, with no maintenance steroids. Of the six patients in the rituximab arm, five developed BPAR in the first 3 months post-transplant compared with one of seven in the control arm. In response to this study, van den Hoogen and Hilbrands [66] report the interim results of a randomized controlled trial of 280 patients comparing rituximab intraoperatively with a placebo. Patients in both arms of the trial also received tacrolimus, MMF and prednisolone. They analysed the first 65 patients who had reached 6-month follow-up and found a relative risk of acute rejection in the rituximab arm of 0.53 (95% confidence interval 0.21–1.32) – the trial is therefore continuing.

Clatworthy *et al.* [65] suggested that the cytokine release syndrome caused by rituximab may enhance T cell activation, thereby increasing acute rejection rates. It is possible that the increased rate of rejection may stem either from the fact that they did not give prolonged steroids, which could protect against the effects of cytokine release, or from

the lack of administration of an IL2 receptor antagonist. The timing of rituximab administration could also be important (although both the other described trials also administer rituximab at the time of transplant). Rituximab in ABOi renal transplantation has generally been given approximately 1 month prior to transplant, allowing time for complete B cell depletion to occur and for any resultant cytokine release to have resolved.

The ongoing randomized controlled clinical trial, RituxiMab INDuction in renal transplantation (ReMIND, NCT01095172 [67]) has been designed to take into account these issues. Only living donor renal transplant recipients are eligible – this allows for planning of the rituximab infusion, which is given 2–4 weeks prior to transplant. In addition, all patients in the trial (both in the rituximab and control arms) receive basiliximab and post-transplant steroids. Participants in the rituximab arm of the trial stop steroids after 1 week.

Rituximab and PTLD

Post-transplant lymphoproliferative disorder (PTLD) is a heterogeneous group of diseases, the majority of which are of B cell origin. Sixty to seventy per cent of B cell PTLD is associated with EBV infection [68]. There is a bi-modal distribution of PTLD presentation relating to EBV status: in one study, median time to diagnosis was 11.5 months in EBV-positive patients and 69 months in EBV-negative patients [69]. PTLD has been reported in 1.2–1.6% of renal transplant recipients [70–72]. According to the European Best Practice Guidelines [73] rituximab is the recommended treatment for CD20+ lymphomas, and in 'the case of diffuse lymphomas or improper response to previous treatment, CHOP [cyclophosphamide, doxorubicin, vincristine and prednisolone] chemotherapy should be used alone or in combination with rituximab' [73]. Rituximab has been demonstrated to be an effective treatment for PTLD [74]. More recently, a phase II trial examining the combination of rituximab followed by CHOP chemotherapy as treatment for PTLD has demonstrated a response to treatment in 90% of patients [75].

It has also been suggested that rituximab may be of use in prophylactic treatment of EBV viraemia prior to the development of PTLD [76]. Twenty (60.6%) of thirty-three EBV seronegative transplant recipients who received a transplant from a seropositive donor and underwent surveillance developed EBV viraemia in the first year post-transplant. Six of these were given rituximab – viraemia resolved in five patients after one dose, and in the sixth patient after two doses. None of these patients subsequently developed PTLD, but 4 of the remaining 14 did develop PTLD ($P = 0.207$). A larger (ideally randomized) trial is needed to confirm these findings.

Risks associated with rituximab use

Rituximab appears generally to be a safe drug, but there have been some concerns relating to its use.

Rituximab and infectious complications

Rituximab may be an effective treatment for EBV viraemia, but concerns have been raised about the possibility of an association between rituximab administration and an increase in other infection rates. Case reports have been published of patients who have developed *Pneumocystis* pneumonia, [77,78], Hepatitis B [79] and CMV disease with bilateral interstitial infiltrates [80] after treatment with rituximab – none of these patients were reported to be on prophylactic therapy at the time of infection.

A number of case series have also been published, with differing conclusions. Some have not found higher rates of infection with rituximab [81–85]. However, other studies have suggested that rituximab is associated with a high rate of infection [86] or an increased rate when compared with patients who did not receive rituximab [18], although these differences have not always been statistically significant [87]. Kamar *et al.* [88] have been quoted widely as demonstrating evidence that rituximab is associated with higher rates of infection. They compared renal transplant patients who received rituximab for any reason (including but not limited to acute and chronic rejection) with a control group who had not received rituximab. The overall rate of infections in the rituximab group was 45.45% and in the control group was 53.88% (albeit over a longer follow-up period). No significant difference was seen in the rate of overall infections or of bacterial infections. Patients in the control group were more likely to have had a viral infection ($P = 0.003$) and patients in the rituximab group were more likely to have had a fungal infection ($P = 0.0005$). The rate of death related to infections was higher in those patients who received rituximab (9.09%) as opposed to those who did not receive rituximab (1.55%, $P = 0.0007$). As noted by Drage *et al.* [89], there are a number of methodological flaws in this study, including that the control group was not directly comparable with the rituximab group, either in time or definition (as the rituximab group were more likely to have been treated for rejection, and received a wide variety of immunosuppressive agents).

Among studies with primary outcomes relating to allograft survival and function, infections have been reported as secondary outcome measures. The randomized controlled trial examining rituximab used in the treatment of acute rejection found no difference in infectious complications between the two groups [56]. Similarly, the randomized controlled trials of rituximab induction also found no difference in infection rates between their two groups [64,66].

Viral and bacterial prophylaxis varies widely, making comparison of infection rates between transplant centres problematic. Another difficulty with assessing the impact of rituximab on infection rates is that, as discussed above, rituximab is often used for desensitization in combination with agents which inhibit or deplete T cells, and as an additional treatment in AMR once other treatments have been unsuccessful. It is therefore being added to an already high immunosuppressive burden. It may well be this overall immunosuppressive effect, including total lymphocyte depletion, rather than any inherent effect of B cell depletion itself, that is the explanation for the finding in some studies of higher rates of infection associated with rituximab. When infections have been assessed in studies, it has either been as a secondary outcome measure in prospective trials, where the trials do not have sufficient power to detect possible differences, or infection rates have been assessed in retrospective studies with a number of confounding factors such as the administration of multiple immunosuppressive drugs and heterogeneous populations. Further studies, designed explicitly to assess the impact of rituximab on infection rates, are required.

Rituximab and thrombocytopenia

Acute thrombocytopenia is a rare, self-limiting complication following rituximab administration, which is unlikely to lead to bleeding [90]. It may be related to the number of pretreatment circulating B cells [91] and the onset of Cytokine Release Syndrome [90].

Rituximab and neutropenia

Rituximab may also be associated with late onset neutropenia (LON) – a low neutrophil count occurring 4 weeks or more after rituximab treatment. In a literature review, Wolach *et al.* [92] identified the incidence of LON to be between 3% and 27% of patients (the studies predominantly were performed in patients treated with rituximab for lymphoma), with LON commencing at a median of 38–175 days after rituximab treatment and lasting for a median of 5–77 days. Only 18 (16.9%) of those patients who developed LON subsequently developed an infectious complication.

The mechanisms underlying the development of LON are not clear, and it has only rarely been seen in solid organ transplantation: Mitsuhata *et al.* have recently reported LON [93] in a patient who had received rituximab for AMR.

Rituximab and progressive multifocal leucoencephalopathy

Progressive multifocal leucoencephalopathy (PML), a demyelinating disease of the central nervous system, results from the reactivation of JC polyoma virus. A retrospective review has identified PML in patients treated with rituximab [94] – the majority had received rituximab as treatment for B cell lymphoproliferative disorders, and all patients had received a number of immunosuppressive agents in addition to rituximab. Ninety per cent of patients with PML died as a result of the disease.

The retrospective review included only one renal transplant recipient who had developed PML (after receiving rituximab for treatment of PTLD). While there are no other reports in the literature of PML developing in renal transplant patients following treatment with rituximab, JC viraemia has been detected: Kamar *et al.* [95] found that four patients of 73 who received rituximab for solid organ transplantation had JC viraemia detected. (All four patients had received rATG, OKT3 or chemotherapy in addition to rituximab.)

Rituximab and cytokine release syndrome

Cytokine release syndrome (an infusion reaction, typically occurring during the first infusion of a new drug, leading to systemic effects such as flu-like symptoms and, rarely, 'severe hypotension, bronchospasm … and even death' [96]) has been associated with administration of rituximab. A small study has suggested that cytokine release syndrome occurs in patients with a high number of B cells [91].There is less risk of developing cytokine release syndrome in renal transplant recipients. In patients with B cell malignancies, the number of CD20+ cells susceptible to rituximab are much greater than in patients with renal failure, who tend to have lower numbers of B cells [97]. The effects of a cytokine release syndrome can be pre-empted by prophylactic administration of paracetamol, steroid and an antihistamine.

Rituximab and cardiovascular complications

Medium to long-term follow-up of participants in one of the randomized controlled trials examining the use of rituximab as induction therapy in renal transplantation has identified a possible effect of rituximab on cardiovascular mortality. At 3-year follow-up [98], 8 of 44 patients assessed had died (one from fungal pneumonia, one from pulmonary carcinoma and six from myocardial infarction); none of 47 patients in the placebo group had died. When examined on an intention-to-treat basis (i.e. using death rates from the original cohorts of 68) the difference in mortality was statistically significant ($P = 0.006$). An increase in cardiovascular reactions has been reported in clinical trials of rituximab use in NHL and CLL, although not in trials in RA [1]. This finding has not previously been reported in renal transplantation, but it does warrant further scrutiny.

B cells and tolerance

There is a theoretical risk that rituximab administration may affect the development of tolerance in renal transplantation, as B cell numbers have recently been found to be increased in patients with operational tolerance [99–101]. Further work is required to investigate any possible impairment of tolerance mechanisms resulting from B cell depletion.

Discussion

Rituximab has become a recognized treatment in induction therapy for ABOi renal transplantation, and is an established and effective treatment for PTLD. The use of rituximab both in HLAi transplantation and the treatment of acute rejection is complex - more evidence is needed, ideally from randomized controlled trials, to establish what effect rituximab has in the management of both these complex areas. Emerging evidence suggests that rituximab may be effective not only in the treatment of established CAMR, but also in prevention. Early results from studies examining rituximab use in antibody compatible renal transplantation suggest that acute rejection rates may be improved, and long-term outcomes from these studies will establish the role of rituximab in reducing late allograft loss.

Some risks are associated with the use of rituximab. Cytokine release syndrome is less of an issue in renal transplantation than in the licensed uses of rituximab, because of the relative paucity of circulating B cells. PML is a rare but serious complication. Concerns have been raised about an increase in infectious complications related to the administration of rituximab – this risk may relate to repeated doses of rituximab or the use of a combination of different immunosuppressive drugs together. Rituximab induction therapy may be related to a higher risk of death from cardiovascular causes – this requires further investigation.

Rituximab is a valuable addition to the pharmacological armoury in renal transplantation. Its use in clinical practice raises a number of questions about the role of B cells in both acute and chronic rejection. Studies designed to answer these questions should not only delineate the best use of rituximab in renal transplantation, but also add to our understanding of the complex interplay between B and T lymphocytes.

Funding

This work was funded/supported by the National Institute for Health Research (NIHR) Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation Trust and King's College London. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health.

Acknowledgements

The authors would like to thank Professor Anthony Dorling for advice during the preparation of this manuscript, and also acknowledge the support of the MRC Centre for Transplantation.

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