Transplant International

Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Impact of low-dose rituximab on splenic B cells in ABO-incompatible renal transplant recipients

Daisuke Toki,[1] Hideki Ishida,[1] Shigeru Horita,[2] Kiyoshi Setoguchi,[1] Yutaka Yamaguchi[3] and Kazunari Tanabe[1]

1 Department of Urology, Tokyo Women's Medical University, Tokyo, Japan
2 Kidney Center, Tokyo Women's Medical University, Tokyo, Japan
3 Department of Pathology, Kashiwa Hospital, The Jikei University of School of Medicine, Chiba, Japan

Keywords
ABO-incompatible renal transplantation, CD20 antigen, desensitization protocol.

Correspondence
Dr Daisuke Toki, 8-1 Kawada-cho, Shinjuku-ku, Tokyo, 162-8666, Japan. Tel.: 81 3 3353 8111; fax: 81 3 3356 0293; e-mail: tokidsk@gmail.com

Received: 29 July 2008
Revision requested: 12 August 2008
Accepted: 2 December 2008

doi:10.1111/j.1432-2277.2008.00821.x

Summary

The purpose of this study was to assess the effect of a low-dose rituximab (RIT) at <375 mg/m^2 on B cells in the spleen and peripheral blood. Five renal transplant recipients received a single dose of RIT at 10, 15, 35, 150, or 300 mg/m^2 3–13 days before transplantation. One patient who received the same immunosuppressive regimen except for RIT was also enrolled as a control. Splenectomy was performed at the time of transplantation in all patients. The B-cell count in the peripheral blood was analysed with a fluorescence-activated cell sorter using anti-CD19 antibodies, and the B cells in the spleen were analysed by immunohistochemistry using anti-CD20 and -CD79a antibodies. All but one dosage (10 mg/m^2) of RIT completely eliminated B cells from the circulation within 30 days. Immunohistochemical examination of the spleen showed a marked reduction of B cells in the white pulps in all five recipients compared with that in the control patient. The observations in this study indicated that RIT has a potent effect of depleting B cells in the spleen and peripheral blood at low-doses of <375 mg/m^2.

Introduction

Rituximab (RIT; Chugai, Tokyo, Japan), a chimeric murine/human monoclonal antibody that binds to the CD20 antigen, has been employed as a component of desensitization protocols in renal transplant recipients, and for treatment of refractory rejection, which did not respond to initial treatment with high-dose steroids therapy, because of its potent B-cell elimination effect; the administration of RIT has provided excellent clinical outcomes [1–6]. Typically, a single dose of RIT at 375 mg/m^2 [2–6], which is known to lead to marked B-cell depletion in the peripheral blood and spleen [7–9], is administered instead of a splenectomy.

Recently, to avoid excessive immunosuppression and the concomitant risk of infection, a tendency has prevailed to reduce the intensity of immunosuppressive treatments. However, administration of low-dose RIT at <375 mg/m^2 has rarely been studied. Moreover, few studies have assessed the optimal dosage of RIT for renal transplant recipients. To our knowledge, only one previous study has examined the effect of low-dose RIT therapy in a renal transplant setting. Vieira *et al.* reported that a single dose of 50 mg/m^2 RIT had the same effect on eliminating circulating B cells as a dose of 375 mg/m^2 [7]. Meanwhile, the splenic response to low-dose RIT has not been clearly established. We believe that elucidating the effect of low-dose RIT on B cells in the spleen is crucial when low-dose RIT is used as a substitute for a splenectomy.

In this article, we report a five-case series in which low-dose RIT therapy in conjunction with splenectomy was employed for renal transplant recipients as a desensitization protocol. The purpose of this study was to assess the impact of a single dose of RIT at <375 mg/m^2 on B cells in the spleen and peripheral blood.

Materials and methods

Patient selection

The desensitization protocol including RIT for renal transplant recipients with donor-specific anti-HLA antibodies (DSHA) and/or anti-blood group antibodies was approved by the clinical ethics committee at Tokyo Women's Medical University in December 2004. We began to use RIT in January 2005. Initially, we administered low-dose RIT at <375 mg/m^2, in combination with splenectomy, to five consecutive ABO-incompatible renal transplant recipients between January and June 2005. After these five patients, we used RIT without splenectomy in the desensitization protocol for ABO-incompatible and highly sensitized recipients. Thus, the five patients enrolled in this series represented our entire investigations into living-donor renal transplantation using low-dose RIT with splenectomy as a desensitization protocol. Written informed consent was obtained from the all patients for renal transplantation using RIT. All subjects had anti-blood group antibodies and DSHA detected using the Luminex single-bead method. As a control, we included another ABO-incompatible recipient who underwent renal transplantation under the same immunosuppressive regimen, except for RIT, in December 2004.

Desensitization protocol and immunosuppression

Seven days prior to transplantation, all patients received a triple-drug immunosuppressive protocol that consisted of tacrolimus (FK), mycophenolate mofetil (MMF), and methylprednisolone (MP), as described previously [10,11]. Briefly, FK (Astellas, Tokyo, Japan) was administered at an initial dose of 0.15 mg/kg/day, and then adjusted to maintain a whole-blood trough level of 8–12 ng/ml for 1–2 months postoperatively, and 7–9 ng/ml thereafter. MMF (Chugai, Tokyo, Japan) was administered at an initial dose of 2000 mg/day and then decreased to 1000–1500 mg/day 1 month postoperatively. MP (Pfizer, Tokyo, Japan) was administered at an initial dose of 20 mg/day; the dosage was increased to 500 mg/day on the day of the operation, and then gradually decreased to 6–8 mg/day within 1–2 months post-transplantation. All patients underwent three or four sessions of double-filtration plasmapheresis (DFPP) before surgery, in addition to splenectomy at the time of transplantation to remove donor-specific anti-blood group antibodies. Basiliximab was administered at a dose of 20 mg/day at the time of transplantation and on postoperative day 4.

Administration of low-dose RIT therapy

A single dose of RIT was administered to each patient 3–13 days before transplantation. The dosages used in each patient are shown in Table 1 [Patient (P) 1, 10 mg/m^2; P2, 15 mg/m^2; P3, 35 mg/m^2; P4, 150 mg/m^2; P5, 300 mg/m^2]. P1 was given an additional 100 mg/m^2 dose of RIT 14 days after the first administration because the B-cell count had started to recover.

Fluorescence-activated cell sorter (FACS) analysis of B cells in the peripheral blood

We used CD19 as a B-cell marker in peripheral blood. Peripheral blood was collected from each subject before RIT administration; at the time of transplantation; and on days 14, 30, 90, 180, and 360 post-administration. A phycoerythrin-conjugated monoclonal antibody against

Table 1. Patient characteristics.

Patient no.	Control	P1	P2	P3	P4	P5
Dosage of RIT (mg/m^2)	0	10*	15	35	150	300
Time between RIT and SPX (days)	–	13	8	4	3	5
Time between RIT and DFPP† (days)	–	3	2	–1	0	–1
Recipient age	67	55	28	42	60	47
Recipient gender	M	F	M	F	F	M
Primary disease	CGN	Gestosis	CGN	IgA	IgA	CGN
Blood type (donor/recipient)	A/B	A/B	B/O	B/O	B/O	A/B
Anti-blood group IgG titer, initial	1:64	1:128	1:16	1:512	1:64	1:8
CDC crossmatch (T/B)	–/–	–/–	–/+	–/+	–/+	–/+
Flow crossmatch (T/B)	–/+	+/+	–/+	–/+	–/+	–/+
Luminex DSA‡ (Class I/Class II)	–/–	+/+	+/+	+/+	–/+	+/–
PRA value (%) (Class I/Class II)	18/3	93/14	91/11	8/9	91/49	88/77
AMR within 3 months	No	Yes	Yes	No	No	No
S-Cr (mg/dl), at 2 years	1.54	1.40	1.45	0.96	0.80	0.97

*P1 was administered an additional 100 mg/m^2 rituximab 14 days after the initial administration.

†Time between RIT and the first session of DFPP (days before DFPP).

‡Donor-specific anti-HLA antibodies were detected by the Luminex single-bead technique.

RIT, rituximab; SPX, splenectomy; M, male; F, female; CGN, chronic glomerulonephritis; T, T-cell crossmatch positive; B, B-cell crossmatch positive; PRA, panel reactive antibody; AMR, antibody-mediated rejection.

Journal compilation © 2008 European Society for Organ Transplantation **22** (2009) 447–454

CD19 (Becton Dickinson, San Jose, CA, USA) was added to whole blood at 4 °C for 30 min. Lysing buffer (BD FACS Lysing Solution®; Becton Dickinson) was added to these samples, which were then allowed to stand at room temperature for 10 min. A lymphocyte gate was set and the purity determined by $CD45^{+}CD14^{-}$ staining (Simultest™Leucogate™CD45/CD14®; Becton Dickinson). CD19-stained peripheral blood cells were fixed and analysed in a FACS (FACSCalibur®; Becton Dickinson) using Simultest™ software (Becton Dickinson).

Immunohistochemistry for identifying B-cell subsets in the spleen

All five patients treated with RIT and the control patient underwent splenectomy at the time of renal transplantation. Each resected spleen was formalin-fixed and paraffin-embedded using standard techniques. For immunohistochemical staining, 3-μm sections were de-paraffinized and endogenous peroxidase activity was blocked with hydrogen peroxide in methanol. Antigen retrieval was carried out by autoclaving the sections at 120 °C for 20 min in citrate buffer (pH 6.0). B cells were identified by staining with mouse antihuman CD20 (Dako, Osaka, Japan) and mouse antihuman CD79a (Dako) antibodies. A mouse antihuman CD138 (Dako) antibody was used to detect plasma cells. These primary antibodies were used to stain three serial sections to evaluate the distribution of each antigen in the same white pulp. The sections were treated with a peroxidase anti-mouse secondary reagent (EnVision™ Detection Reagent; Dako) for 30 min at room temperature.

Quantitative analysis of B cells in the splenic white pulps

To assess the effect of RIT on B cells in the spleen, we performed a quantitative analysis of the B-cell splenic follicles. CD20- and CD79a-stained areas, which represent B-cell splenic follicles, in 80 consecutive white pulps were quantified using image analysis software (Win Roof®; Mitani Corporation, Fukui, Japan) by a pathologist who was unaware of the other findings. The average value of 80 consecutive positively stained areas was then calculated.

Results

Patient characteristics

Patient characteristics are summarized in Table 1. The patients consisted of three women and three men aged

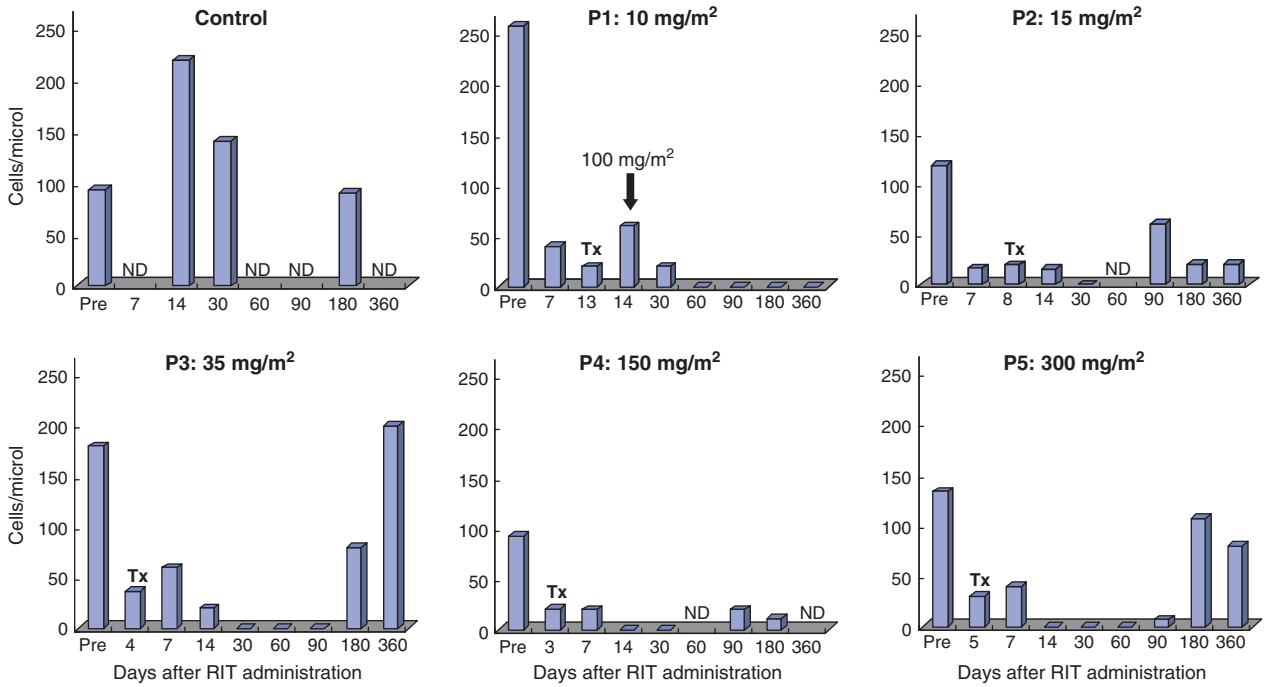

Figure 1 Fluorescence-activated cell sorter (FACS) analysis of B cells in the peripheral blood pre- and post-administration of rituximab. The number of B cells relative to the time of rituximab administration is demonstrated. FACS analysis was performed using anti-CD19 antibodies. The dosage of rituximab is shown at the top of each panel. Control patient was not administered rituximab. P1 was administered an additional 100 mg/m^2 rituximab 14 days after the initial infusion. (Tx, Transplantation; ND, not done.)

between 28 and 67 years. Three recipients were blood group A-incompatible and the other three patients were group B-incompatible. The range of initial anti-blood group IgG titers was between 1:8 and 1:512. The five patients who received RIT had both positive flow-crossmatch and DSHA detected by Luminex single bead. Four out of the five patients had high panel reactive antibody (PRA) values (PRA > 80%).

FACS analysis of B cells in the peripheral blood

A single dose of RIT eliminated B cells from the peripheral blood in all but one patient (P1, 10 mg/m^2; Fig. 1). In P1, partial recovery of the B-cell count was noted 14 days after the first RIT treatment; thus, the patient was administered an additional 100 mg/m^2 dose on the same day. This second dose eliminated B cells for about 1 year. The time required to eliminate B cells was longer in P2 (15 mg/m^2) and P3 (35 mg/m^2) than in P4 (150 mg/m^2) and P5 (300 mg/m^2) (30 vs. 14 days). B cells on the day of transplantation were considerably suppressed, but were not completely depleted in all the recipients (P1: 20 cells/μl; P2: 19 cells/μl; P3: 37 cells/μl; P4: 21 cells/μl; and P5: 30 cells/μl). Partial recovery of the B-cell count was noted in P2 on day 90, but the population remained below the pre-RIT level until day 360 (pre-RIT: 118 cells/μl; day 90: 63 cells/μl; and day 360: 20 cells/μl). B-cell depletion was almost sustained in P3–P5 until day 90 (P3: 0 cells/μl; P4: 20 cells/μl; and P5: 7 cells/μl). In P4, B-cell depletion was still maintained on day 180 after administration of RIT but an apparent increase in B-cell number was noted in P3 and P5 at 180 days (P3: 80 cells/μl; P4: 11 cells/μl; and P5: 107 cells/μl).

Immunohistochemical identification of B-cell subsets in the spleen

Representative immunohistochemical findings of a white pulp from four spleens (control, P1, P3, and P5) are shown in Fig. 2. In the control spleen, there were many B cells in white pulps and the distribution of CD20$^+$ and CD79a$^+$ cells was very similar. Meanwhile, CD138$^+$ cells were rare in the white pulps. The administration of RIT at 10 mg/m^2 in P1 did not result in adequate B-cell

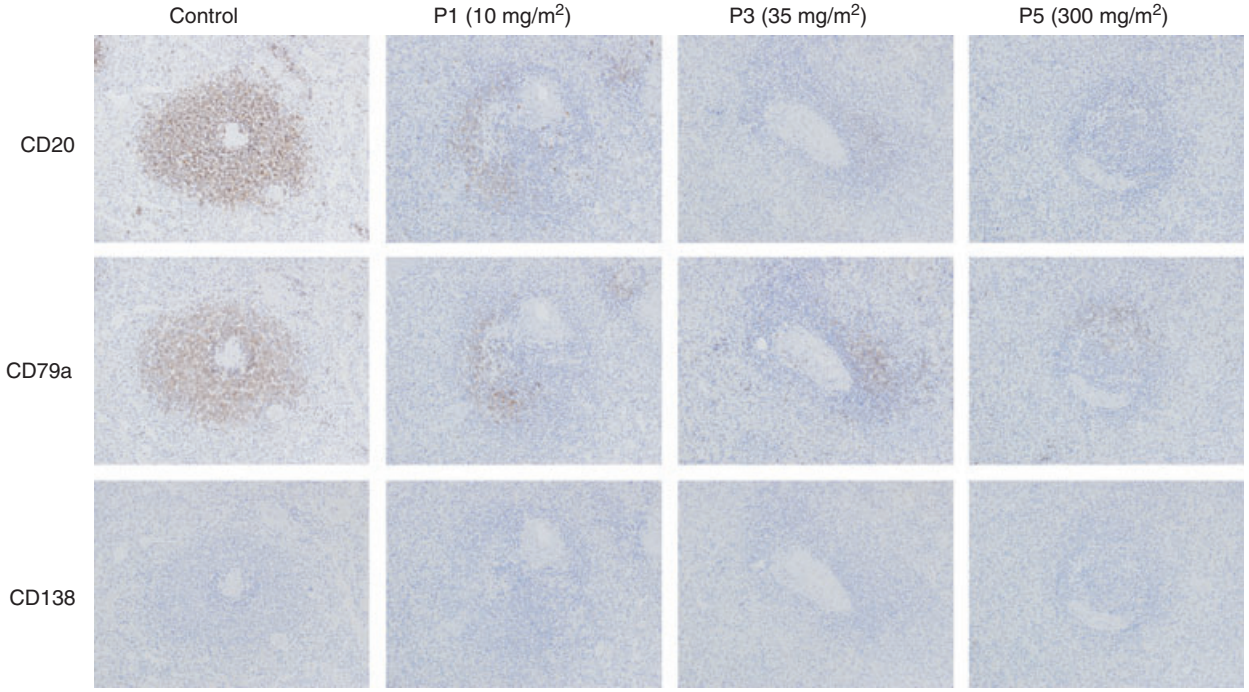

Figure 2 Imuunohistochemistry for identifying B-cell subset. Representative white pulps of four patients are presented. Three serial sections were used for immunohistochemistry (CD20, CD79a and CD138). The dosage of rituximab in each patient is shown at the top of the figure. Control patients did not receive rituximab. CD138-positive plasma cells were rarely observed in the white pulps of all the patients. The distribution of CD20- and CD79a-positive B cells was almost the same in the white pulps of control and P1. However, the number of B cells was apparently reduced in P1. CD20-positive B cells almost completely disappeared from the white pulps but only a few CD79a-positive B cells were residual in the white pulps in P3 and P5.

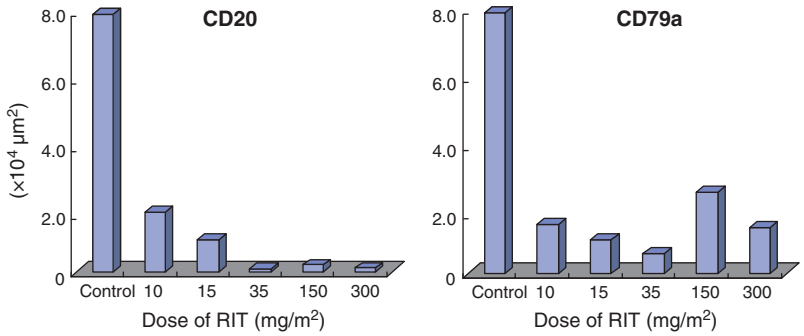

Figure 3 Quantitative analysis of B cells in the white pulps. Eighty consecutive areas of CD20- or CD79a-staining which represent B-cell splenic follicles in the white pulps of each patient were measured and the average was calculated. CD20-positive B cells were rarely observed even in the patient who received rituximab at a dose of $\geqq$35mg/m^2 (left panel). Meanwhile, CD79a-positive B cells in the white pulps were residual in all patients who received rituximab. However, the areas of CD79a-positive B cells in the patients who received rituximab were apparently reduced compared with that in the control patient (right panel).

depletion; the distribution of CD20$^+$ and CD79a$^+$ cells in the spleen was also similar in this case. However, in P3 and P4 (data not shown), and P5, we observed a difference in the distribution of CD20$^+$ and CD79a$^+$ cells. In these patients, our examination of serial sections showed that CD20$^+$ cells were rare in the white pulps. However, residual CD79a$^+$ cells were observed in the same white pulps and were not concordant with CD138$^+$ plasma cells (Fig. 2).

Quantitative analysis of B cells in the splenic white pulps

We quantitatively evaluated the size of the B-cell splenic follicles after RIT administration. The mean sizes of both the CD20$^+$ and CD79a$^+$ areas decreased apparently after RIT administration in all patients compared with the control (Fig. 3). P1 and P2 showed residual CD20$^+$ cells, but P3–P5 showed total elimination of CD20$^+$ cells. In contrast, CD79a$^+$ cells were not completely eliminated from the spleen in any of the patients, although the population did decrease compared with that in the control patient.

Clinical outcome

The clinical outcome for each patient is shown in Table 1. Both P1 and P2 developed acute antibody-mediated rejection (AAMR) on postoperative day 17, while the remaining three patients did not. Two years after surgery, the serum creatinine concentration remained at normal levels in P3–P5. We encountered no severe complications related to the administration of RIT. Two patients (P1 and P4) developed cytomegalovirus infection; P1 manifested a high fever, while P4 showed no clinical manifestations. Both episodes were treated successfully with ganciclovir.

Anti-blood group IgG titers and DSHA

The anti-blood group IgG titer at the time of transplantation decreased to ≤1:32 in all patients. The postoperative anti-blood group IgG titers were not elevated above the levels at the time of transplantation for 1 year after transplantation in all patients, regardless of the dosage of RIT. DSHA disappeared in P1 and P3, while DSHA was detected in serum obtained P2, P4 and P5 within 6 months after transplantation.

Discussion

Previous studies have revealed that administration of a single dose of RIT prior to transplantation completely depletes circulatory B cells [7,8]. Genberg *et al.* pointed out that a single dose of RIT at 375 mg/m^2 along with FK, MMF/azathioprine and MP in renal transplant recipients led to the complete elimination of B cells from the peripheral blood, and that the B-cell population remained suppressed for several years [8]. Vieira *et al.* revealed in their dose-escalation study that a single dose of RIT, even at 50 mg/m^2, depleted B cells from the peripheral blood as effectively as a single dose of 375 mg/m^2 [7]. In their study, the B-cell count remained suppressed for at least 1 year after treatment, although recovery began 6 months post-treatment. Consistent with these results, we found that dosages as low as 15 mg/m^2 caused complete circulatory B-cell depletion in renal transplant recipients. B-cell recovery began at 3–6 months after RIT administration, which was faster than B-cell recovery after 375 mg/m^2 RIT [8]. However, because AAMR typically occurs within 3 months after transplantation, the effects of low-dose RIT therapy seem to be appropriate in terms of the desensitization protocol [2,12].

In this study, we focused on the response of B cells in the spleen to RIT because in many institutions, RIT has been used as a substitute for splenectomy as a component of the desensitization protocol. A few studies have indicated that RIT induces significant B-cell depletion in the spleen of humans and monkeys [1,9,13]. However, all the evidence in humans was based on dosages of 375 mg/m^2 or over. Sawada *et al.* reported that four doses of RIT at 375 mg/m^2 completely eliminated CD20$^+$ B cells from the spleen in a renal transplant recipient [1]. Ramos *et al.* showed that a single dose of RIT at 375 mg/m^2, combined with plasmapheresis and intravenous immunoglobulin therapy, depleted most B cells, both CD20$^+$ and CD79a$^+$ cells, from the spleen [9]. Meanwhile, to our knowledge, the effect of low-dose RIT on the spleen has not been previously reported. In this study, low-dose infusion of RIT led to a marked reduction in the B-cell population in the spleen. We could not directly compare our results with those of Ramos *et al.* [9] because they used a semi-quantitative method based on intensity and distribution to evaluate the B-cell population in splenic follicles; whereas we used a quantitative method based on their distribution. However, the effect of low-dose RIT on splenic follicles appears to be similar to that of RIT administered at 375 mg/m^2. The semi quantitative method of Ramos *et al.* [9] revealed that a dose of 375 mg/m^2 RIT resulted in grade 1–2 staining in B-cell splenic follicles (0, absent; 1, weak; 2, low moderate; 3, high moderate; 4, intense), which suggests that almost, but not quite all the B cells were depleted from the spleen, consistent with our own results.

Note that serial sections of the spleen showed a difference between CD20 and CD79a staining after the administration of RIT. CD79a is usually used as a B-cell marker in immunohistochemical studies; while, it is known to be expressed on both B cells and plasma cells [14]. Therefore, a possibility exists that the CD79a$^+$CD20$^-$ cells in the splenic follicles in our study represented plasma cells. However, CD20 and CD79a showed almost the same distribution and CD138$^+$ plasma cells were rarely observed in the splenic follicles of the control patient. Based on these observations, we assumed that the CD79a$^+$CD20$^-$ cells in the splenic follicles were not plasma cells but residual B cells, after administration of RIT.

Two interpretations of CD79a$^+$CD20$^-$ B cells are conceivable. In the first, since CD79a expression is known to precede CD19 or CD20 expression in the earliest differentiation events of B-cell ontogeny [15,16], it is possible that the CD79a$^+$CD20$^-$ immature B cells, which are not susceptible to RIT, were residual in the spleen. Previous studies [8,9] have also shown that there were more residual CD79a$^+$ cells than CD20$^+$ cells in the spleen, lymph nodes, and kidney. The role of CD79a$^+$CD20$^-$ B cells in the rejection process is unclear, but CD79a$^+$CD20$^-$ B cells have the potential to differentiate into mature B cells and plasma cells in the spleen and may cause antibody-mediated rejection. The second interpretation is that RIT might have masked CD20 epitopes on B cells. Previous studies [17,18] have demonstrated the possible internalization of CD20 epitopes after RIT administration; it has also been suggested that RIT can mask CD20 epitopes, resulting in false negative expression of CD20 in FACS analysis of B cells in peripheral blood, as FACS analysis can detect only extracellular epitopes. However, B cells express not only extracellular CD20 epitopes but also intracellular CD20 epitopes. Therefore, the CD20 antibody should detect intracellular CD20 epitopes in an immunohistochemical analysis. However, it is possible that the intensity of CD20 staining might have been attenuated, if the extracellular epitopes were covered and masked by RIT.

The timing between infusion and transplantation is as important as the dosage of RIT. The effect of RIT on B cells in the peripheral blood is rather rapid, with detectable B-cell elimination within a few days [7,8]. In contrast, circulatory B-cell elimination was not observed until 14–30 days after RIT administration in this study. A possible reason for this delay was the relatively low dose employed. However, this does not explain the difference between our results and those reported by Vieira *et al.* [7], in which low-dose RIT eliminated peripheral B cells within 2 days. Another conceivable reason for the difference between the two studies is that we subjected our patients to DFPP, which might have attenuated the effect of RIT on B cells; although no one has studied whether DFPP can remove RIT from the circulation.

Although a single dose of RIT at 375 mg/m^2 is generally accepted as being adequate in a renal transplant setting [2–6], little evidence exists to indicate whether this is actually the most appropriate dosage for renal transplant recipients. Furthermore, the long-term complications of RIT administration remain unknown. As a complication, late-onset neutropenia has been reported after administration of 150 mg/m^2 RIT in a renal transplant recipient [19,20]. The authors of that study suggested that 375 mg/m^2 may be an excessive dosage for renal transplant recipients [19,20]. Segev *et al.* reported favorable outcomes in four cases of ABO-incompatible renal transplantation without splenectomy or RIT [21]. In the current tendency to reduce the intensity of immunosuppression, we propose that low-dose RIT therapy would be a valid option for desensitization or rescue therapy for refractory rejection. Since 2007, we have employed RIT at a single dose of 200 mg/body instead of splenectomy as a component of the desensitization protocol in ABO-incompatible recipients, and have achieved good results. With respect

Journal compilation © 2008 European Society for Organ Transplantation **22** (2009) 447–454

to patients covered by our preliminary data, we experienced only one episode of AAMR in 19 ABO-incompatible renal transplantations with this low-dose RIT protocol without splenectomy, between January 2007 and June 2008 (unpublished data).

We experienced two cases of AAMR on postoperative day 17. Each patient received very-low-dose RIT at 10 and 15 mg/m^2 respectively. Neither patient had elevated anti-blood group antibody titers at the time they developed AAMR. DSHA disappeared in P1; while, P2 still possessed DSHA after the transplantation. Accordingly, we could not conclude whether the anti-blood group antibodies or anti-HLA antibodies contributed to the development of AAMR in the two recipients. The intervals between the administration of RIT and the development of AAMR were 30 and 25 days respectively, during which time the circulating levels of B cells were depleted in the two patients (Fig. 1). This suggests that B-cell elimination from both the peripheral blood and spleen is not sufficient to suppress the development of AAMR in some high-risk recipients such as this cohort. The B cells in the lymph nodes and bone marrow are thought to be less susceptible to RIT than those in the peripheral blood [13]. In this study, we did not examine B cells in the lymph nodes and bone marrow, but it is possible that compared with other dosages, the very-low-dose of RIT could not adequately deplete B cells in these compartments, and this might have led to the development of AAMR. The important findings in this article were that both patients received a conventional immunosuppressive regimen before application of RIT, including FK, MMF, MP, plasmapheresis, and splenectomy. This means that some high-risk recipients need more potent immunosuppressive protocols compared with the conventional protocols.

We acknowledge that the small number of patients in the present case series was a limitation in this study. We also understand that extrapolating our results to the general renal transplant population is difficult. However, we believe that the evidence from this small series may be a clue to further studies on how to use low-RIT because when RIT is employed as a substitute for splenectomy, comprehending the status of the spleen after administration of RIT is essential. Another limitation of this study is that the timing between the infusion of RIT and splenectomy differed among the patients, at 3–13 days. Nevertheless, the encouraging findings in this study are that a single dose of 35 mg/m^2 RIT had almost the same impact on B cells in the spleen and peripheral blood as 300 mg/m^2 RIT, even though splenectomy was performed only 4 days after RIT administration. If splenectomy had been carried out 14 days or more after RIT administration, more splenic B cells would have been eliminated because at least 14 days were required to eliminate B cells completely from the circulation after RIT administration. In addition, because we did not standardize the time interval between RIT administration and the first session of DFPP, the effect of RIT according to each dose might not have been evaluated equally.

In conclusion, we showed that administration of low-dose RIT markedly reduced B-cell numbers in the spleen and peripheral blood. Further studies are required to determine the most appropriate dosage and timing of RIT administration in renal transplant recipients.

Authorship

DT: designed study, wrote paper. HI: edited manuscript; cared for patients at transplant surgery. SH: performed immunohistochemical staining for spleen. KS: designed study. YY: diagnosed renal biopsies. KT: edited manuscript.

Funding

I have no financial relationships to disclose.

Acknowledgements

The authors thank Hideki Nakayama, Miyuki Furusawa and Tsutomu Ishizuka for their excellent technical assistance with immunohistochemistry and flow cytometry. The authors also thank Dr. Izumi Yamamoto at the Division of Kidney and Hypertension, Department of Internal Medicine, The Jikei University of School of Medicine, for his critical advice of immunohistochemical analysis.

References

1. Sawada T, Fuchinoue S, Teraoka S. Successful A1-to-O ABO-incompatible kidney transplantation after a preconditioning regimen consisting of anti-CD20 monoclonal antibody infusions, splenectomy, and double-filtration plasmapheresis. *Transplantation* 2002; **74**: 1207.
2. Gloor J, DeGoey S, Pineda A, *et al.* Overcoming a positive crossmatch in living-donor kidney transplantation. *Am J Transplant* 2003; **3**: 1017.
3. Sonnenday C, Warren D, Cooper M, *et al.* Plasmapheresis, CMV hyperimmune globulin, and anti-CD20 allow ABO-incompatible renal transplantation without splenectomy. *Am J Transplant* 2004; **4**: 1315.
4. Tydén G, Kumlien G, Genberg H, Sandberg J, Lundgren T, Fehrman I. ABO incompatible kidney transplantations without splenectomy, using antigen-specific immunoadsorption and rituximab. *Am J Transplant* 2005; **5**: 145.
5. Becker Y, Becker B, Pirsch J, Sollinger H. Rituximab as treatment for refractory kidney transplant rejection. *Am J Transplant* 2004; **4**: 996.

6. Lehnhardt A, Mengel M, Pape L, Ehrich J, Offner G, Strehlau J. Nodular B-cell aggregates associated with treatment refractory renal transplant rejection resolved by rituximab. *Am J Transplant* 2006; **6**: 847.
7. Vieira C, Agarwal A, Book B, *et al.* Rituximab for reduction of anti-HLA antibodies in patients awaiting renal transplantation: 1. Safety, pharmacodynamics, and pharmacokinetics. *Transplantation* 2004; **77**: 542.
8. Genberg H, Hansson A, Wernerson A, Wennberg L, Tydén G. Pharmacodynamics of rituximab in kidney allotransplantation. *Am J Transplant* 2006; **6**: 2418.
9. Ramos E, Pollinger H, Stegall M, Gloor J, Dogan A, Grande J. The effect of desensitization protocols on human splenic B-cell populations in vivo. *Am J Transplant* 2007; **7**: 402.
10. Setoguchi K, Ishida H, Shimmura H, *et al.* Analysis of renal transplant protocol biopsies in ABO-incompatible kidney transplantation. *Am J Transplant* 2008; **8**: 86.
11. Ishida H, Miyamoto N, Shirakawa H, *et al.* Evaluation of immunosuppressive regimens in ABO-incompatible living kidney transplantation–single center analysis. *Am J Transplant* 2007; **7**: 825.
12. Toma H. ABO-incompatible renal transplantation. *Urol Clin North Am* 1994; **21**: 299.
13. Schröder C, Azimzadeh A, Wu G, Price J, Atkinson J, Pierson R. Anti-CD20 treatment depletes B-cells in blood and lymphatic tissue of cynomolgus monkeys. *Transpl Immunol* 2003; **12**: 19.
14. Mason D, Cordell J, Brown M, *et al.* CD79a: a novel marker for B-cell neoplasms in routinely processed tissue samples. *Blood* 1995; **86**: 1453.
15. Ryan D, Kossover S, Mitchell S, Frantz C, Hennessy L, Cohen H. Subpopulations of common acute lymphoblastic leukemia antigen-positive lymphoid cells in normal bone marrow identified by hematopoietic differentiation antigens. *Blood* 1986; **68**: 417.
16. Dworzak M, Fritsch G, Fröschl G, Printz D, Gadner H. Four-color flow cytometric investigation of terminal deoxynucleotidyl transferase-positive lymphoid precursors in pediatric bone marrow: CD79a expression precedes CD19 in early B-cell ontogeny. *Blood* 1998; **92**: 3203.
17. Jilani I, O'Brien S, Manshuri T, *et al.* Transient down-modulation of CD20 by rituximab in patients with chronic lymphocytic leukemia. *Blood* 2003; **102**: 3514.
18. Cragg M, Bayne M, Illidge T, Valerius T, Johnson P, Glennie M. Apparent modulation of CD20 by rituximab: an alternative explanation. *Blood* 2004; **103**: 3989; author reply 90-1.
19. Mitsuhata N, Fujita R, Ito S, Mannami M, Keimei K. Delayed-onset neutropenia in a patient receiving rituximab as treatment for refractory kidney transplantation. *Transplantation* 2005; **80**: 1355.
20. Mitsuhata N, Fujita R, Ito S, Mannami M, Kojima K. Is administration of rituximab at 375 mg/m without splenectomy appropriate for ABO-incompatible renal transplant? *Am J Transplant* 2005; **5**: 3019. author reply 20.
21. Segev D, Simpkins C, Warren D, *et al.* ABO incompatible high-titer renal transplantation without splenectomy or anti-CD20 treatment. *Am J Transplant* 2005; **5**: 2570.

