CLINICAL RESEARCH

Rituximab Administration within 6 Months of T Cell-Depleted Allogeneic SCT is Associated with Prolonged Life-Threatening Cytopenias

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The monoclonal anti-CD20 antibody Rituximab (RTX) is increasingly used in allogeneic stem cell transplantation (SCT) to treat lymphoproliferative disorders and chronic graft-versus-host disease (GVHD). RTX administration can be complicated by delayed and prolonged neutropenia, but the mechanism is unclear. We report the occurrence of profound cytopenias following RTX given in the conditioning regimen or early after T cell-deplete SCT to treat B cell lymphoproliferative disorders or chronic GVHD (cGVHD). Between 2006 and 2009, 102 patients (median age: 43 years, range: 13-68 years), received a myeloablative matched-sibling T cell-deplete SCT for lymphoid or myeloid hematologic disorders. Neutropenia occurring within 4 weeks of treatment developed in 16 of 17 patients given RTX within the first 190 days after SCT. Fourteen patients developed severe neutropenia (count <0.5 K/μL) lasting up to 10 months and 12 required hospitalization to treat severe neutropenic infections. Six of the 14 patients died of infection complicating GVHD treatment. Recovery of lymphocytes and immunoglobulins was also delayed, with a significantly lower absolute lymphocyte counts (ALC) at 9 months and 12 months post-SCT compared to patients with cGVHD not treated with early RTX ($P < .02$). In contrast, patients receiving RTX 1 year after SCT experienced only moderate neutropenia 3 to 5 months after treatment lasting 10 to 20 days while maintaining absolute neutrophil count (ANC) $>1.0 \times 10^9$/L. Although RTX rapidly controlled cGVHD, we conclude that its administration early after T cell-deplete SCT is associated with prolonged profound and life-threatening cytopenias, and should be avoided.

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INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (SCT) offers the possibility of a curative treatment for malignant and nonmalignant hematologic diseases. However, SCT is frequently complicated by graft-versus-host disease (GVHD), which remains a major cause of transplant-related morbidity and mortality (TRM). The anti-CD20 chimeric monoclonal antibody Rituximab (RTX) given prior to, or during, conditioning for T cell-replete SCT has been reported to decrease acute GVHD, and chronic GVHD (aGVHD, cGVHD), and may decrease TRM [1–3]. Because of these promising results, RTX has been increasingly used to treat cGVHD [4].

RTX induces response rates in about two-thirds of patients with cGVHD. Response varies by organ, with an estimated response rate of 60% for cGVHD of the skin compared to approximately 30% for cGVHD of the gastrointestinal (GI) tract, liver, or lung [5]. Apart from acute infusion reactions, RTX is well tolerated. However, late adverse effects are being identified with increased frequency. Late-onset neutropenia is estimated to occur in up to 35% of patients treated for B cell malignancies in the non-SCT setting [6]. Thrombocytopenia (platelets <75 K/μL) and anemia (hemoglobin <10 g/dL) have also been reported, with an incidence of approximately 12% and 6%, respectively [7].

Since 2006, we have used RTX in the early transplant period after myeloablative SCT, either as part of the conditioning regimen for B cell malignancies, or to treat emerging cGVHD. Although patients

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with cGVHD responded well to RTX, all patients who received RTX within 6 months after SCT had a high risk of developing severe cytopenias. Here, we describe the clinical outcome of RTX-treated patients and discuss the possible etiology of RTX-induced cytopenias in this patient population.

MATERIALS AND METHODS

Patients and Controls

Between February 2004 and April 2009, 102 consecutive patients underwent a T cell-depleted SCT from an HLA-identical sibling in 3 successive National Heart, Lung and Blood Institute (NHLBI) institutional review board-approved protocols (04-H-0112, 06-H-0248, and 07-H-0136). Patients and donors provided written informed consent before enrolling in the transplantation protocol.

All patients received a conditioning regimen of fludarabine 125 mg/m^2 over 5 days, fractionated total-body irradiation (TBI) 12 Gy (4.0 Gy if over 55y) in 8 fractions over 4 days, followed by cyclophosphamide 120 mg/kg over 2 days. All transplants were depleted of T lymphocytes with the Isolex system (protocol 04-H-0112), or with the Miltenyi CliniMacs system (Miltenyi Biotec Inc., Auburn, CA) (protocols 06-H-0248 and 07-H-0136) as previously described [8,9]. In protocols 04-H-0112, 06-H-0248 patients received an infusion of donor lymphocytes between days 60 to 90 after SCT. In protocol 07-H-0136 patients received 5×10^6 selectively depleted CD3$^+$ cells/kg on day 0, as previously described [10].

Only patients surviving 6 months or longer after SCT were included in the analysis to allow sufficient time for the development of cGVHD, and to exclude patients that experienced early deaths because of unrelated causes. Of the 95 patients surviving 6 months or longer after SCT, 17 received RTX within 6 months of SCT. Twenty-eight patients developed cGVHD but did not receive RTX early after SCT (4 received RTX 1-7 years after SCT), 18 of whom received an SCT prior to the use of RTX for treatment of cGVHD at our institution and were therefore considered the historic controls for this analysis. Fifty patients did not develop cGVHD and did not receive RTX at any time after SCT. Chronic GVHD was diagnosed and graded consistent with NIH consensus criteria [11].

GVHD Prophylaxis

All patients received low-dose (LD) cyclosporine (CsA) (target plasma level, 100-200 μg/mL), starting on day −4 and continuing according to protocol to day +21 or day 90 after SCT. CsA was reinitiated and continued for approximately 3 months after donor lymphocyte infusions (DLIs) given by protocol or to treat incipient rejection as documented by falling counts and falling donor T cell chimerism. CsA was continued or reinitiated if cGVHD developed, and patients were treated off protocol for cGVHD refractory to CsA and prednisone.

Infection Prophylaxis and Treatment

Standard prophylaxis against infection included fluconazole and bactrim given for at least 6 months after transplantation, and twice weekly surveillance for cytomegalovirus (CMV) DNA by polymerase chain reaction (PCR). Treatment of infections was in accordance with the Guidelines for Management in Allogeneic Hematopoietic Stem Cells Transplant Recipients published by the Center for Disease Control (CDC) (http://www.cdc.gov/mmwr/preview/mmwrhtml/rr4910a1.htm). Granulocyte colony stimulating factor (G-CSF) was administered in all cases to maintain and absolute neutrophil count (ANC) >500/μL.

RTX Administration and Response Criteria

RTX given in the first 6 months after SCT was administered by intravenous infusions of RTX (375 mg/m^2 per infusion) at 2 to 4 weekly intervals posttransplant to treat cGVHD (15 patients), Epstein-Barr virus (EBV) lymphoproliferative disease (1 patient), and autoimmune hemolytic anemia (1 patient). Three patients with B cell malignancies that received RTX for treatment of cGVHD also received RTX immediately prior to, or as part of, the SCT conditioning regimen. In addition, 4 patients received RTX 1 to 7 years post-SCT at the same dose and schedule to treat cGVHD. Response of cGVHD to RTX was assessed 1 month after the last infusion. Complete response (CR) was defined as resolution of all manifestations of cGVHD in involved organs. A partial response (PR) was defined as an improvement in 1 or more involved organ without any progression or new organ involvement. Resistance was defined as no response or worsening cGVHD requiring alternative therapy.

Statistical Analysis

Survival was measured to the last contact date or death. Univariate and multivariate analyses were performed using Cox proportional-hazard regression model, including all factors associated with a *P*-value <.2 by univariate analysis, and all factors statistically different among the early RTX and other groups (P <.10). A stepwise backward procedure was then used with a cutoff significance level of .05 to remove factors from the model. *P*-values are 2 sided, with a type I error rate fixed at .05. Statistical analyses were performed with SPSS 15.0 and Prism 4 software.

RESULTS

cGVHD Response to RTX

Eight of the 15 patients who received RTX for treatment of cGVHD during the first 6 months after SCT experienced complete remission of all cGVHD symptoms shortly after RTX administration, 4 patients developed a PR but continued to require first-line cGVHD treatment, and 3 patients required further second-line treatment (Table 1). All 4 patients receiving RTX 1 to 7 years after SCT responded, 3 with CR and 1 with PR.

Blood Counts and Immunoglobulin Levels during the First 12 Months after SCT

Onset of cytopenias occurred a median of 4 weeks after administration of RTX and led to a significant difference in blood counts between the 3 groups (early RTX, cGVHD without early RTX, no cGVHD) during the first year after SCT (Figure 1A-C). Patients treated within 6 months after SCT with RTX experienced lower absolute neutrophil counts at 6 months and 9 months ($P < .01$). These patients also experienced lower absolute lymphocyte counts throughout the first posttransplant year ($P < .01$ at 6, 9, and 12 months), a lower median platelet count at 6 months after SCT ($P < .01$), and lower immunoglobulin levels for up to 2 years post-SCT. The median level of IgM was lowest at 9 months after SCT, and occurred 12 months or later for IgG and IgA (Figure 1E). Clinically significant anemia requiring red blood cell (RBC) transfusions was infrequently noted. However, no difference in erythropoeitic activity was noted between the 3 groups as represented by equivalent absolute reticulocyte counts during the first year after SCT, and anemia requiring RBC transfusions occurred infrequently (Figure 1D).

Timing of RTX Administration and Severity of Lymphopenia Correlates with Duration of Neutropenia

The earlier RTX was given after SCT, the longer was the duration of cytopenias. Patients receiving their first dose of RTX at least 20 weeks after SCT experienced relatively short episodes of cytopenias with a mean duration of moderate neutropenia (ANC <1000 cells/μL) of 1.3 months, and of severe neutropenia (ANC <500 cells/μL, G-CSF dependence) of 0.6 months. In contrast, patients receiving RTX between 10 and 20 weeks after SCT experienced prolonged neutropenia (mean duration 3.3 months of moderate neutropenia, and 1.7 months of severe neutropenia). Patients treated with RTX within 10 weeks of SCT experienced the longest cytopenias (mean duration of 13.7 months of moderate neutropenia, and 5 months of severe neutropenia) (Figure 2). In total, 16 of the

Table 1. Outcomes of Patients Treated with RTX within 30 Weeks of SCT

Diagnosis	Week of First Dose of RTX	No. of doses of RTX within 30 Weeks of SCT	Bacterial Infections	Fungal Infections	Autoimmune Disorders	Outcome	Days Survival post-SCT
MCL	−8	3	Cellulitis	None	T-LGL	Alive	815
MCL	−5	7	Cellulitis, pneumonia, septicemia	Zygomycosis pneumonia	GI overlap syndrome	Died fungal pneumonia	959
CLL	0	5	Pneumonia, cellulitis, septicemia	Zygomycosis pneumonia	GI overlap syndrome	Died fungal pneumonia	503
ALL Ph+	10	2	Pneumonia, septicemia	*S. prolificans*		Died ICH	214
AML	12	3	Cellulitis, septicemia	None		Died of Relapse	218
ALL Ph+	13	2	Pneumonia, septicemia	Aspergillus pneumonia	GI overlap syndrome	Died fungal pneumonia	443
AML	15	3	None	None		Alive	577
MDS	15	2	Cellulitis, septicemia	Aspergillus + Zygomycosis pneumonia	T-LGL	Died fungal pneumonia	414
AML	16	3	None	None		Alive	1039
AML	16	4	Cellulitis	None		Alive	430
MDS/AML	19	3	Cellulitis, bowel perforation, septicemia	None	ITP	Alive	759
AML	20	3	Cellulitis	None		Died Idiopathic Pneumonitis	195
ALL Ph+	22	2	Pneumonia	None		Alive	703
ALL Ph−	22	2	Cellulitis	None		Alive	1362
APL	23	3	None	None		Alive	1564
APL	23	4	Pneumonia, septicemia	Apergillus pneumonia	NK-LGL,GI overlap syndrome	Died fungal pneumonia	648
CML	27	2	Cellulitis	None		Alive	528

RTX indicates Rituximab; SCT, stem cell transplantation; AML, acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; APL, acute promyelocytic leukemia; CML, chronic myelogenous leukemia; CLL, chronic lymphocytic leukemia; NHL, non-Hodgkin Leukemia; MDS, myelodysplastic syndrome; Ph+, Philadelphia chromosome; NK or T-LGL, NK or T cell-large granulocytic leukemia; GI, gastrointestinal.

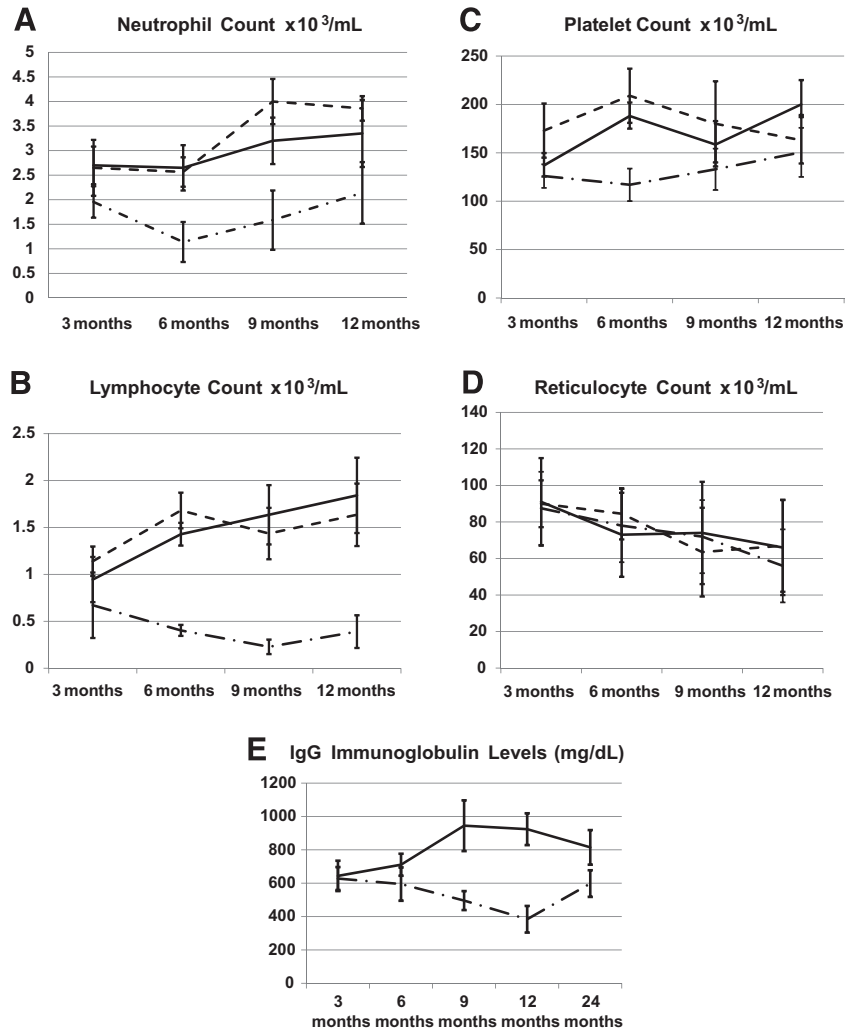

Figure 1. (A-E) Median absolute peripheral counts during the first year after SCT, and median absolute IgG immunoglobulin levels during the first 2 years after SCT. – – – No cGVHD; —— cGVHD without early RTX; – • – Early RTX.

17 patients developed severe neutropenia, 5 of which experienced only a minimal responsive to prolonged G-CSF administration. All 4 patients receiving RTX 1 to 7 years after SCT experienced only moderate neutropenia 3 to 5 months after treatment lasting 10 to 20 days while maintaining ANC $>1.0 \times 10^9$/L.

RTX administration was associated with a profound nadir in absolute lymphocyte counts (ALC) occurring within 4 weeks after the administration of the last dose. Patients developing an ALC nadir less than the median of 140 lymphocytes/μL experienced the longest duration of neutropenia (ANC <1000/μL, median 7

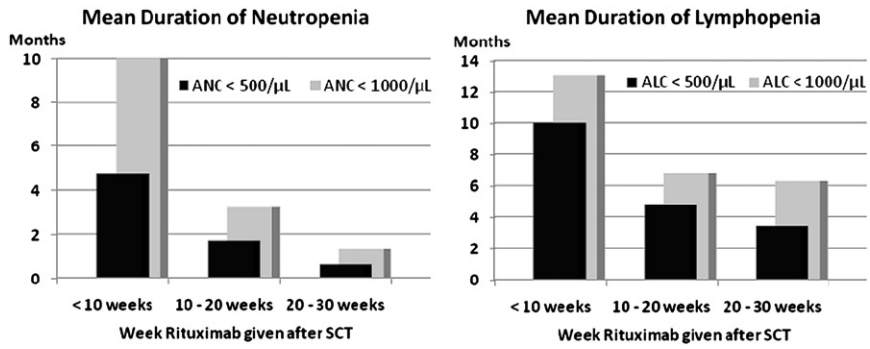

Figure 2. Timing of RTX administration and duration of neutropenia. Mean duration of cytopenias experienced by patients when treated with their first dose of RTX within various time periods in relation to SCT. Duration of cytopenias inversely correlated with the time interval between RTX administration and SCT; patients that received RTX within 10 weeks of SCT experiencing the longest duration of neutropenia and lymphopenia.

Table 2. Patients with cGVHD Who Received RTX within 30 Weeks of SCT

Disease	Indication for RTX	Week of First Dose of RTX	Week of Chronic GVHD Onset	Organs Involved with Chronic GVHD	cGVHD Severity (NIH Consensus Criteria)	Treatment of Chronic GVHD Prior to RTX	No. of Doses within 30 weeks of SCT	Other Second-Line Treatments after RTX
MCL	NHL, GVHD	−8	13	Skin, Joints	Mild	Pred, CsA	3	
MCL	NHL, GVHD	−5	14	Skin, Joints, GI	Moderate	Pred, CsA, MMF	7	Daclizumab and Infliximab, PhotoPh
CLL	CLL , GVHD	0	19	Skin, Liver, GI	Moderate	Pred, CsA, Tacro, MMF	5	
ALL Ph+	GVHD	10	10	Skin, Liver, GI	Moderate	Pred, CsA	2	
AML	GVHD	12	12	Skin	Mild	Pred, CsA	3	
ALL Ph+	GVHD	13	12	Skin, GI	Moderate	Pred, CsA, MMF	2	Daclizumab and Infliximab
AML	GVHD	15	13	Skin, Joints	Moderate	Pred, CsA, MMF	3	
MDS	GVHD	15	13	Skin, Joints	Moderate	Pred, CsA	2	
AML	EBV LPD	16	*	*	*	*	3	
AML	GVHD	16	14	Skin, Joints, Lung	Moderate	Pred, CsA, MMF	4	Imatinib
MDS/AML	GVHD	19	16	Skin, Liver, GI	Moderate	Pred, CsA, Tacro, MMF	3	
AML	GVHD	20	19	Skin, Lung	Moderate	Pred, CsA, MMF	3	
ALL Ph+	GVHD	22	16	Skin, Joints	Moderate	Pred, CsA, MMF	2	
ALL Ph−	GVHD	22	16	Skin, Joints	Moderate	Pred, CsA, MMF	2	Imatinib
APL	GVHD	23	18	Skin, Joints, Liver	Moderate	Pred, CsA, Tacro, MMF	3	
APL	GVHD	23	14	Skin, Joints, Liver	Moderate	Pred, CsA, Tracro, MMF	4	Daclizumab and Infliximab
CML	AIHA	27	*	*	*	*	2	

RTX indicates rituximab; SCT, stem cell transplantation; AML, acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; APL, acute promyelocytic leukemia; CML, chronic myelogenous leukemia; CLL, chronic lymphocytic leukemia; NHL, non-Hodgkin leukemia; MDS, myelodysplastic syndrome; Ph+, Philadelphia chromosome; EBV, Epstein-Barr virus; AIHA, autoimmune hemolytic anemia; PhotoPh, Photopheresis; CsA, cyclosporine; Tacro, tacrolimus; Pred, prednisone; GVHD, graft-versus-host disease.
*Received RTX for PTLD and AIHA.

months versus 1.5 months, $P < .01$). Death because of infection only occurred in this group.

Outcomes

All patients received anti-Candida prophylaxis with fluconazole initiated at time of SCT, and all patients were switched to voriconazole for anti-Aspergillus prophylaxis at the time of cGVHD diagnosis with introduction of steroid therapy. Fourteen of the 17 patients treated with RTX within 6 months of SCT developed recurrent bacterial infections, with superficial cellulitis, bacterial pneumonia, and septicemia occurring in the majority of cases (Table 1). Additionally, 6 patients developed significant fungal infections and 5 died with invasive fungal pneumonia (2 Apergillus, 3 Zygomyces). Four patient developed evidence of autoimmune disorders; 2 with T cell large granular lymphoproliferative disease (T-LGL), with >30% $CD8^+CD57^+$ cytotoxic T cells by flow cytometry of peripheral blood, 1 patient with natural killer (NK)-LGL (>90% NK dominance of lymphocytes in bone marrow specimen), and 1 patient with immune thrombocytopenic purpura diagnosed clinically and by increased megakaryocytes in the bone marrow biopsy. Three patients developed aGVHD overlap syndrome of the gastrointestinal tract, which occurred up to 2.5 years after SCT and was documented by biopsy.

Patients with cGVHD treated with RTX early after SCT had higher TRM (64% versus 18%, $P = .03$) when compared to all other patients with cGVHD. To exclude selection bias and the possibility that RTX was used only to treat the most recalcitrant forms of cGVHD, we compared results with historic cGVHD controls not treated with RTX at our institution (Tables 2, 3, and 4). Again, patients with cGVHD treated with RTX within 6 months of SCT had significantly higher TRM compared with the cGVHD historic controls (64% versus 17%, $P = .02$), suggesting that the early administration of RTX increased the risk of TRM independent of cGVHD status. In univariate and multivariate analysis only RTX administration was associated with increased TRM (hazard ratio [HR] = 5.54, 95% confidence interval [CI] 1.12-27.2, $P = .03$, Figure 3).

DISCUSSION

cGVHD is a major cause of SCT-related morbidity and mortality, and steroid dependent or resistant patients have a worse prognosis. RTX is effective in the treatment of steroid refractory cGVHD and has been used increasingly early after SCT to treat cGVHD in addition to its use in controlling lymphoproliferative disorders. This report highlights a potential risk of RTX administration within 6 months of T cell-depleted SCT.

Although cytopenias are known to occur following RTX administration, severe RTX-induced cytopenias have not been described in the context of allogeneic SCT [6,12]. Previous reports describe only limited cytopenias when RTX is administered early after T cell-replete SCT [13,14]. A possible explanation for the worse outcomes in our patients receiving RTX is that the risk of cytopenias is related to the T cell

Table 3. Historical Controls, Patients with cGVHD Who Did Not Received RTX Early after SCT

Disease	Week of cGVHD Onset	Organs Involved with cGVHD	cGVHD Severity (NIH Consensus Criteria)	Treatment of cGVHD	Other Second-Line Treatments
AML	16	Skin, Joints	moderate	Pred, CsA, MMF	
AML	31	Skin, Liver, Eyes	moderate	Pred, CsA, MMF	
CLL	23	Skin, Joints	mild	Pred, CsA	
AML	19	Skin, Eyes	mild	Pred, CsA	
ALL	14	Liver	moderate	Pred, CsA, Tacro	
MDS	16	Skin, Liver, Mouth	moderate	Pred, CsA, Tacro	
CML	22	Skin, Joints	moderate	Pred, CsA, MMF	
AML	14	Skin, Lungs	moderate	Pred, CsA	
AML	14	Skin, Joints	moderate	Pred, CsA	
AML	16	Skin, Joints	mild	Pred, CsA	
ALL	13	Skin	moderate	Pred, CsA	
CML	15	Skin, Mouth, Eyes	moderate	Pred, CsA, MMF	
AML	10	Skin, Liver, GI	severe	Pred, CsA, Tacro	Daclizumab and Infliximab
AML	14	Skin, Joints, Lung	moderate	Pred, CsA, MMF	
AML	16	Skin, Joints	moderate	Pred, CsA, MMF	Imatinib
AML	12	Skin, Liver	moderate	Pred, CsA, Tracro	Daclizumab and Infliximab
MDS	15	Skin, Renal	moderate	Pred, CsA	
AML	26	Skin, Joints, Eyes	moderate	Pred, CsA, MMF	

AML indicates acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; APL, acute promyelocytic leukemia; CML, chronic myelogenous leukemia; CLL, chronic lymphocytic leukemia; NHL, non-Hodgkins Leukemia; MDS, myelodysplastic syndrome; CsA, cyclosporine; Tacro, tacrolimus; Pred, prednisone; GVHD, graft-versus-host disease; RTX indicates Rituximab.

depletion of the graft, which delivers only a limited quantity of B cells to the recipient. In our patients whose allografts were manipulated to remove T lymphocytes, $<1 \times 10^3$ B cells/kg were infused at the time of transplantation, rendering them profoundly B cell depleted. Additionally, the reduced inoculum of T cells in the lymphopenic milieu at time of SCT may have contributed to the risk of cytopenias by predisposing our patients to clonal expansions of small numbers of residual $CD8^+$ T cells, which further enhanced the immune imbalance induced by RTX [15,16].

The mechanism of increased TRM with early RTX administration after T cell-deplete SCT in our patient population appears multifactorial involving impaired B-lymphocyte, neutrophil, and T cell function. Although the half-life of RTX ranges from 2 to 3 weeks, detectable levels may persist 3 to 6 months after administration [17,18]. Previous studies have demonstrated that RTX administration within 1 year prior to SCT significantly impaired B cell reconstitution and resulted in a significant B cell deficiency lasting up to 2 years after T cell-replete SCT [19]. Similar to our experience, the duration of B cell deficiency was also inversely correlated with the time interval between RTX administration and SCT, indicating a profound B cell depleting effect of RTX when given in close proximity to SCT. Compared to other patients, including those with cGVHD, we found that early RTX recipients had reduced levels of immunoglobulins and an increased risk of infection. It was notable that early RTX recipients had lower median neutrophil counts in the first 9 months after SCT compared with other patients (including those developing cGVHD who did not receive early RTX). Clearly, the prolonged neutropenia contributed to the infectious complications encountered after early RTX administration despite the aggressive use of G-CSF, which provided only temporary improvement of the neutrophil count. The profound lymphopenia

Table 4. Univariate Analysis of Risk Factors for TRM in Patients with cGVHD

Covariable	cGVHD Not Receiving Early RTX	Patients with cGVHD Treated with RTX <6 Months of SCT	Univariate Analysis *P*-Value
RTX administration	0/18 patients	15/15 patients	.02
Age	Median 34 (range: 19-48)	Median 41 (range: 30-58)	.09
Reason for SCT			
AML	12	5	.73
ALL	1	4	
CML	2	0	
NHL	1	3	
MDS	2	2	
Disease risk			
Low	7	1	.69
Intermediate	2	9	
High	9	12	
Severity of cGVHD prior to RTX administration			
Mild	6	3	.18
Moderate	12	11	
Severe	0	0	
Number of organs involved			
1	10	2	.80
2	4	7	
3+	4	5	
DLI given	11	3	.10

RTX indicates Rituximab; cGVHD, chronic graft-versus-host disease; SCT, stem cell transplantation; AML, acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myelogenous leukemia; NHL, non-Hodgkin leukemia; MDS, myelodysplastic syndrome; DLI, donor lymphocyte infusion.

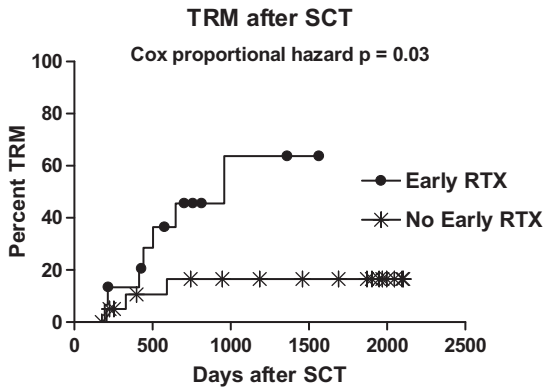

Figure 3. Administration of RTX to treat cGVHD within 6 months of SCT was associated with higher TRM.

accompanying the neutropenia in our patients was striking, and was associated with more severe cytopenias and worse outcome.

The neutropenia and lymphopenia following RTX administration is not easily explained. The cytopenias we observed appear distinct from the effects of cGVHD because neutrophil, platelet, and lymphocyte counts were significantly lower than in patients developing cGVHD who did not receive RTX early after SCT. In mice, short-term B cell depletion reduces expansion, activation, and effector cell differentiation of $CD4^+$ T cells, whereas $CD8^+$ activation is not affected [20]. A decreased conversion of $CD4^+$ T cells from the naïve to a central memory phenotype is also observed, suggesting that a significant functional and maturational deficiency exists in the $CD4^+$ compartment in the absence of B cell and antigen-specific $CD4^+$ T cell interactions. Similarly in humans, lower $CD4^+$ and $CD4^+Foxp3^+$ regulatory T cells are noted in patients with diseases of defective B cell differentiation, and are associated with an inversion of the $CD4^+/CD8^+$ ratio and a higher incidence of autoimmune disorders [21]. Consequently, the immune dysregulation occurring after B cell depletion suggests an immune-mediated mechanism in the pathogenesis of RTX-associated cytopenias. A recent report demonstrates that patients who develop RTX-associated late onset neutropenia have inverted $CD4^+/CD8^+$ ratios and more pronounced T cell expansions. Proliferation of T-large granular lymphocytes with increased expression and secretion of Fas and Fas ligand was also noted, and bone marrow evaluation demonstrated dyshematopoiesis with extensive hypoplasia of the granulocytic series, consistent with findings seen in immune-mediated T-LGL [22]. The occurrence of LGL and ITP in 4 patients treated with RTX supports the possibility that RTX induced clonal expansion of autoreactive $CD8^+$ T cells, which suppressed neutrophil production. We are now searching for expanded LGL $CD8^+$ T cell clones in all neutropenic RTX recipients.

In conclusion, RTX is a powerful immune modulatory agent that is effective in the treatment of cGVHD. However, prolonged and life-threatening cytopenias can occur when RTX is administered within 6 months of T cell-depleted SCT. Although the mechanism remains unclear, the increased incidence of auto- and alloimmune diseases after RTX administration suggests immune dysregulation. We recommend avoiding RTX administration within 6 months of T cell-deplete SCT to minimize the risk of life-threatening cytopenias and other immune mediated adverse events.

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