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REACTIVATION OF LATENT VIRUSES IN INDIVIDUALS RECEIVING RITUXIMAB FOR NEW ONSET TYPE 1 DIABETES

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Abstract

Background—Rituximab has been successfully used as an experimental therapy in different autoimmune diseases. Recently, a double-blind placebo-controlled phase-2 study in early onset type 1 diabetes showed that rituximab delayed progression of the disease. However, like with any immunosuppressive therapy, there is a concern of opportunistic viral reactivations with the use of rituximab, including herpes and polyomaviruses.

Objectives—To study the incidence of new infections and reactivations with BK, JC, Epstein-Barr and cytomegalovirus (BKV, JCV, EBV and CMV) in T1D participants in the phase-2 rituximab study.

Study Design—Subjects received 4 weekly doses of rituximab (N=57) or placebo (N=30) during the first month of study. Blood samples obtained at weeks 0, 12, 26, 56 and 78 were assayed for CMV, EBV, BKV and JCV by real-time DNA PCR and serology.

Results—EBV reactivations were diagnosed by PCR in 25% of placebo, but none of rituximab recipients ($p<0.01$). There were no episodes of CMV viremia in either treatment group. BKV viremias were significantly more common in the rituximab recipients (9%) compared with placebo controls (0, $p<0.01$). No JCV reactivations were detected in this study, but among 6 rituximab and 2 placebo recipients who seroconverted for JCV during the study, only one rituximab recipient had detectable viremia. All infections were asymptomatic.

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Conclusions—Four doses of rituximab administered to individuals with early onset T1D decreased the incidence of asymptomatic EBV reactivations, as predicted by the rituximab-mediated elimination of memory B-cells, but increased the frequency of asymptomatic viremias caused by polyomaviruses.

Keywords

Rituximab; Type 1 diabetes; Epstein Barr virus; JC virus; BK virus; Cytomegalovirus

BACKGROUND

Rituximab is a molecularly engineered, chimeric murine/human anti-human CD20 monoclonal antibody. While rituximab was initially approved for treatment of B cell non-Hodgkin's lymphoma, it has been successfully used in many different antibody-mediated or antibody-associated diseases such as chronic refractory idiopathic thrombocytopenic purpura (ITP)[1], myasthenia gravis[2], and rheumatoid arthritis[3, 4]. Recent data suggest that even classically considered antibody-mediated diseases, such as ITP, might be T cell-mediated, in which case the beneficial effect of rituximab might result from elimination of antigen-presenting B cells[5].

The pathophysiology of type 1 diabetes (T1D) most likely requires the presentation of beta cell antigens to T cells within lymph nodes. The antigen reactive T cells then migrate to the pancreas where autoimmune destruction of the beta cells occurs. B cells may play a crucial role as antigen presenting cells in T1D. A recent double-blind placebo-controlled phase 2 study of rituximab in early onset T1D showed a delay of disease progression in the treatment group[6]. Further studies indicated that rituximab attenuated beta-cell loss, although it did not decrease proliferative responses to beta cell antigens[7].

Rituximab eliminates mature circulating B cells for up to nine months. Severe and even fatal cases of hepatitis B (HBV) and other viral reactivations were described after rituximab treatment in combination with other chemotherapeutic or immunosuppressive agents[8–10]. Among herpesviruses, fatal varicella zoster virus (VZV)[11] and cytomegalovirus (CMV) reactivations[12, 13] were described. Recently, a review of FDA reports, manufacturer's database and publications revealed 57 cases of progressive multifocal leukoencephalpathy (PML) in HIV-negative patients treated with rituximab with a case fatality rate of 90%. The median time from the first rituximab dose to PML diagnosis was 16 months (range =10–90 months) and the median time from the last dose of rituximab to PML diagnosis was 5.5 months (range =0.3–66 months)[14]. Another review of 64 cases of serious viral infections associated with rituximab treatment found that the median interval from the start of rituximab treatment to diagnosis of viral opportunistic infections was 5 months (range =1–20 months). The most common agents of viral reactivations were HBV (39%, N=25), CMV (23%, N=15) and VZV (9%, N=6). Of the patients with HBV infections, 13 (52%) died from hepatic failure. Among the 39 viral infections other than HBV, 13 had a fatal outcome[15].

OBJECTIVES

We evaluated the incidence and outcome of primary infections and reactivations of EBV, CMV, BKV and JCV in rituximab and placebo recipients with early onset T1D enrolled in a previously described study[6] over 78 weeks of follow-up.

STUDY DESIGN

Subjects

Of 87 participants between 8 and 40 years old, 57 were randomly assigned to receive rituximab (Table 2). Four 375mg/m^2 doses of rituximab were administered on days 1, 8, 15 and 22[6]. Blood samples obtained at weeks 0, 12, 26, 56 and 78 were assayed for EBV, CMV, BKV and JCV circulating DNA and antibodies.

PCR Assays

EBV and CMV real-time PCR were performed on whole blood as previously described[16]. Viral DNA was extracted from 200 μl of previously frozen whole blood using the MagNA Pure apparatus (Roche). Ten μl of extracted DNA were added to 10 μl of DNA PCR master mix containing LightCycler FastStart DNA reaction mix (Roche), primers, (0.5 μM each), probes (0.2 μM each) and MgCl2 (3.5 mM). PCR primers and probes are listed in Table 1. The reaction was allowed to develop over 45 cycles of denaturation, annealing, and elongation in the LightCycler apparatus (Roche). The specificity of the PCR product was confirmed by its melting curve generated at the end of the amplification process. The lower limit of detection (LLD) of both assays was 100 copies/ml. The dynamic range (linear portion of the curve) spanned from 500 to 1,000,000 DNA copies/ml.

For BKV and JCV real-time PCR, viral DNA was extracted from 200ul serum using the MagNA Pure apparatus Roche. Five μl of extracted DNA were added to 15μl of DNA PCR master mix containing LightCycler FastStart DNA reaction mix (Roche), MgCl2 (3mM for BKV and 4mM for JCV), primers (0.5mM each) and probes (0.25mM each). Primers and probes PCR primers and probes are listed in Table 1. The reaction developed over 45 cycles in the LightCycler apparatus. The specificity of the PCR products was confirmed by their melting curves. A PCR run was considered valid if all the controls including high and low positives, negative and extracted performed within their pre-specified ranges. The number of DNA copies/reaction tube was calculated by comparison with DNA standards (Advanced Biotechnology Inc.) containing a pre-defined number of copies. The LLDs were 600 DNA copies/ml for BKV and 100 copies/ml for JCV. The dynamic ranges were 2000–10,000,000 for BKV and 500–1,000,000 copies/ml for JCV.

Serology Assays

Anti-EBV antibodies were measured using the semi-quantitative Diamedix Immunosimplicity®-IS VCA IgG Test (Diamedix) and the qualitative MERIFLUOR® EBV VCA IgM IFA (Meridian) as per manufacturers' instructions. Anti-CMV antibodies were measured using the semi-quantitative Diamedix Immunosimplicity®-IS CMV IgG Test (Diamedix) and the qualitative Diamedix Immunosimplicity®-IS CMV IgM Capture Test as per manufacturer's instructions.

IgG, IgM and IgA anti-BKV and JCV capsids were measured using BK and JC virus-like particles (VLPs)-based ELISA. 96-well microtiter plates Nunc were coated overnight at 4°C with 25 ng of VLPs produced as previously described[17]. Plates were incubated for 2h at room temperature with 300μl blocking buffer consisting of 0.5% polyvinyl alcohol Sigma and Blocker Casein Thermoscientific in PBS; washed with PBS containing 0.05% Tween 20; and incubated with duplicate serum samples diluted 1:200 in blocking buffer for IgG and 1:100 for IgA and IgM assays. After 1h at 37°C on a microplate shaker, bound IgG, IgA or IgM were detected using goat anti-human IgG, IgA or IgM, respectively, conjugated with horseradish peroxidase (Southern Biotech) and 2,2′-azino-di-3-ethylbenzthiazoline-6-sulfonate (Kirkegaard & Perry) colorimetric substrate. The plates were read at 405nm in an automated microtiter plate reader (Molecular Devices) with a reference wavelength of

490nm. A cut off value for seropositivity was defined as the optical density >mean + 3 S.D. of the reactivity of serum samples from a low prevalence population (children ≤2 years of age) after excluding outliers.

Definitions of primary, recent, past infections and reactivations/reinfections by serology

Past infection was defined by positive IgG and negative IgM and/or IgA results at entry; recent infection by positive IgG and IgM and/or IgA at entry; primary infection by negative IgG at entry followed by positive IgG and/or IgM and/or IgA at any time during the study; reactivation/reinfection by past infection and a ≥2-fold increase in IgG titer or new positive IgM and/or IgA during the follow-up.

Statistical analysis

The frequency of acute, recent, past infections, reactivations/reinfections and PCR-measured viremia was compared between placebo and treated subjects with Fisher's Exact Test. P-values <0.05 were considered statistically significant.

RESULTS

EBV Infections

At baseline, 39 of 78 (50%) subjects with serology data had evidence of past infection, including 27 of 51 (53%) rituximab and 12 of 27 (44%) placebo recipients. During the 78 weeks of follow-up, 2 placebo recipients acquired primary infection diagnosed by seroconversion. There was no evidence of reactivation/reinfection by serology. However, EBV viremia was detected in 3 baseline-seropositive placebo recipients at a total of 4 visits. The viral loads were 250 to 11,970 EBV DNA copies/mL and all episodes were asymptomatic. There were no EBV viremias in the rituximab group. The frequency of EBV viremias was significantly higher among placebo compared with rituximab recipients $p<0.01$ (Table 3).

CMV Infections

At baseline, 20 of 78 (26%) subjects had past infection, including 16 of 51 (31%) rituximab and 4 of 27 (15%) placebo recipients. During the study, 2 (13%) rituximab and 1 (25%) placebo recipients had a ≥2-fold increase in IgG on ≥1 occasion suggesting reactivation/reinfection. There were no episodes of CMV viremia in either group. All reactivations/reinfections were asymptomatic (Table 3).

BKV Infections

At baseline, 64 of 82 (78%) subjects had past infection, including 43 of 52 (83%) rituximab and 21 of 30 (70%) placebo recipients. Sixteen subjects, 8 in each treatment group, had active or recent infection at baseline. During the study, 1 (3%) placebo and none rituximab recipients acquired primary infection. Seven subjects, 5 (12%) rituximab and 2 (10%) placebo recipients, had serologic evidence of BKV reactivation/reinfection. BKV viremia was detected in 4 (9%) baseline-seropositive rituximab, but in none of the placebo recipients. The viral loads ranged between 850 and 7,550 DNA copies/ml. All four subjects were baseline-seropositive. However, none of them showed serologic evidence of reactivation. Statistical analysis showed that subjects who received rituximab were significantly more likely to have BKV viremia than placebo controls ($p<0.01$). All the serologic reactivations/reinfections and the viremic episodes were asymptomatic (Table 3).

JCV Infections

At baseline, 20 of 82 subjects (24%) had past infection, including 16 of 52 (31%) rituximab and 4 of 30 (13%) placebo recipients. Three subjects (6%) had evidence of active or recent infection at baseline. All were in the rituximab group. During the study, 8 subjects acquired primary infection: 6 (17%) in the rituximab and 2 (8%) in the placebo group. One rituximab recipient with primary infection had self-limited JCV viremia with a viral load of 7,800 DNA copies/ml. All primary infections were asymptomatic. None of the subjects had evidence of reactivation by serology or PCR (Table 3).

DISCUSSION

The rates of reactivation/reinfection for the herpes and polyomaviruses in this study were generally low and all episodes were asymptomatic. This is in contrast with previous reports of severe viral infections after rituximab infusion. This study had a limited number of subjects in the rituximab-treated group at risk for CMV or JCV reactivations (16 each) and samples were collected at relatively long intervals. However, the length of follow-up was adequate for detecting viral opportunistic infections, since previous studies reported this type of complications at a median of 5 to 16 months after treatment initiation[14]. There are a few characteristics of our study that may explain the absence of viral complications: 1) administration of a small number (N=4) of rituximab infusions; 2) lack of additional immunosuppressive agents; 3) relatively mild, if any, immunocompromised status of the early onset T1D subjects.

The frequency of BKV viremias was significantly higher in rituximab compared with placebo recipients and occurred in 4 subjects seropositive at baseline indicating that the viremias represented viral reactivations/reinfections. However, reactivations/reinfections diagnosed by changes in antibody titers were equally common in the rituximab and placebo groups. Taken together, these data suggest that rituximab treatment increases the incidence of active BKV replication during reactivations/or reinfections. BKV has been associated with interstitial tubular nephritis and hemorrhagic cystitis in solid organ and hematopoietic stem cell transplant recipients, respectively[18]. Other end-organ diseases such as pneumonia and encephalitis have been ascribed to BKV, but they are much less frequent[19]. In hosts immunocompromised due to transplantation, the likelihood of BKV-associated symptoms increases with the BKV DNA titer in serum and/or urine, with thresholds of 10,000 and 1,000,000 DNA copies/mL, respectively. Urine was not tested in this study, but plasma BKV loads were <10,000 DNA copies/mL, which is consistent with the asymptomatic characteristics of the BKV viremias in this study.

JCV reactivations have the potential of leading to PML, a serious and currently untreatable disease. In this study, we did not observe any JCV reactivations, but the number of subjects seropositive at baseline was relatively low: 16 in the rituximab and 4 in the placebo groups. Of note, the low prevalence of JCV infections was consistent with the age distribution of our subjects. There were 6 subjects in the rituximab treatment group who had serologic evidence of primary infection during the study. All were asymptomatic and only one viremia was detected in this group. Although the number of JCV-seropositive subjects in this study was too small to draw definitive conclusions, our findings suggest that administration of 4 doses of rituximab in individuals with early onset T1D might not increase the incidence of JCV reactivations. However, we cannot rule out that more prolonged administration of rituximab for T1D prophylaxis might increase the incidence of JCV reactivations.

As expected, none of the rituximab recipients had EBV viremia, because rituximab eliminates the memory B cells, which are the main reservoir for EBV latency and reactivation. In contrast, 25% of the placebo controls had EBV viremia. In this study, we

used EBV DNA measurements as a surrogate of viremia based on our previous findings that most of the EBV DNA detected in whole blood is accounted by actively replicating virus both in immunocompromised and immunocompetent hosts{Kroll, #16}. The frequency of EBV viremias in controls was similar to the one observed in placebo-treated T1D subjects enrolled in other TN studies[20] and did not significantly vary with the age of the subjects. We did not study local reactivations in the oropharynx or genital tract. It is interesting to note that the suppressive effect of rituximab on EBV reactivation lasted for at least 18 months after the last rituximab infusion.

CMV reactivations/reinfections were detected by serology only. They were equally frequent in the two treatment groups. Viremias were not detected, but local reactivations in the oropharynx or urogenital tract were not studied and may have been missed. Although severe CMV infection was described in patients treated with rituximab-containing immunosuppressive regimens, individuals with early onset T1D may not be at high risk of systemic dissemination of reactivated CMV.

Before this study, there was a concern that serology might not detect primary infections or reactivations/reinfections in rituximab recipients, due to an attenuation of primary and recall antibody responses. In a previous study, we showed that rituximab recipients respond poorly to primary immunization during the phase of maximal B cell depletion and that responses are later recovered[21]. Responses to booster doses of vaccines were present, but somewhat attenuated compared with placebo recipients. However, in this study, serology identified 6 primary JCV infections and multiple reactivations of CMV and BKV in the rituximab recipients. Furthermore, there were no episodes of viremia in seronegative subjects that would have been another indication that primary infections might not result in seroconversions.

Overall, this study demonstrated that a 4-dose course of rituximab, which was previously shown to decelerate progression of early onset T1D, was not associated with symptomatic primary infections or reactivations of latent viruses, but increased the frequency of viremias associated with BKV reactivations. Additional studies of polyomaviruses are warranted in patients who receive prolonged rituximab monotherapy for the treatment or prophylaxis of T1D.

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References

1. Zaja F, Iacona I, Masolini P, Russo D, Sperotto A, Prosdocimo S, et al. B-cell depletion with rituximab as treatment for immune hemolytic anemia and chronic thrombocytopenia. Haematologica. 2002; 87:189–95. [PubMed: 11836170]

2. Zaja F, Russo D, Fuga G, Perella G, Baccarani M. Rituximab for myasthenia gravis developing after bone marrow transplant. Neurology. 2000; 55:1062–3. [PubMed: 11061276]
3. Edwards JC, Cambridge G. Sustained improvement in rheumatoid arthritis following a protocol designed to deplete b lymphocytes. Rheumatology (Oxford). 2001; 40:205–11. [PubMed: 11257159]
4. Leandro MJ, Edwards JC, Cambridge G. Clinical outcome in 22 patients with rheumatoid arthritis treated with b lymphocyte depletion. Ann Rheum Dis. 2002; 61:883–8. [PubMed: 12228157]
5. Olsson B, Andersson PO, Jernas M, Jacobsson S, Carlsson B, Carlsson LM, et al. T-cell-mediated cytotoxicity toward platelets in chronic idiopathic thrombocytopenic purpura. Nat Med. 2003; 9:1123–4. [PubMed: 12937414]
6. Pescovitz MD, Greenbaum CJ, Krause-Steinrauf H, Becker DJ, Gitelman SE, Goland R, et al. Rituximab, b-lymphocyte depletion, and preservation of beta-cell function. N Engl J Med. 2009; 361:2143–52. [PubMed: 19940299]
7. Herold KC, Pescovitz MD, McGee P, Krause-Steinrauf H, Spain LM, Bourcier K, et al. Increased t cell proliferative responses to islet antigens identify clinical responders to anti-cd20 monoclonal antibody (rituximab) therapy in type 1 diabetes. J Immunol. 187:1998–2005. [PubMed: 21775681]
8. Niitsu N, Hagiwara Y, Tanae K, Kohri M, Takahashi N. Prospective analysis of hepatitis b virus reactivation in patients with diffuse large b-cell lymphoma after rituximab combination chemotherapy. J Clin Oncol. 28:5097–100. [PubMed: 20837949]
9. Perceau G, Diris N, Estines O, Derancourt C, Levy S, Bernard P. Late lethal hepatitis b virus reactivation after rituximab treatment of low-grade cutaneous b-cell lymphoma. Br J Dermatol. 2006; 155:1053–6. [PubMed: 17034541]
10. Zachou K, Dalekos GN. Hepatitis b re-activation with rituximab therapy: Treat the patient not the disease. Liver Int. 31:277–9. [PubMed: 21281426]
11. Bermudez A, Marco F, Conde E, Mazo E, Recio M, Zubizarreta A. Fatal visceral varicella-zoster infection following rituximab and chemotherapy treatment in a patient with follicular lymphoma. Haematologica. 2000; 85:894–5. [PubMed: 10942955]
12. Sharma M, Moore J, Nguyen V, Van Besien K. Fatal cmv pneumonitis in a lymphoma patient treated with rituximab. Am J Hematol. 2009; 84:614–6. [PubMed: 19676117]
13. Suzan F, Ammor M, Ribrag V. Fatal reactivation of cytomegalovirus infection after use of rituximab for a post-transplantation lymphoproliferative disorder. N Engl J Med. 2001; 345:1000. [PubMed: 11575282]
14. Carson KR, Evens AM, Richey EA, Habermann TM, Focosi D, Seymour JF, et al. Progressive multifocal leukoencephalopathy after rituximab therapy in hiv-negative patients: A report of 57 cases from the research on adverse drug events and reports project. Blood. 2009; 113:4834–40. [PubMed: 19264918]
15. Aksoy S, Harputluoglu H, Kilickap S, Dede DS, Dizdar O, Altundag K, et al. Rituximab-related viral infections in lymphoma patients. Leuk Lymphoma. 2007; 48:1307–12. [PubMed: 17613758]
16. Kroll J, Li S, Levi M, Weinberg A. Lytic and latent ebv gene expression in transplant recipients with and without post-transplant lymphoproliferative disorder. J Clin Virol. 52:231–5. [PubMed: 21900040]
17. Viscidi RP, Rollison DE, Viscidi E, Clayman B, Rubalcaba E, Daniel R, et al. Serological cross-reactivities between antibodies to simian virus 40, bk virus, and jc virus assessed by virus-like-particle-based enzyme immunoassays. Clin Diagn Lab Immunol. 2003; 10:278–85. [PubMed: 12626455]
18. Hirsch HH. Polyomavirus bk nephropathy: A (re-)emerging complication in renal transplantation. Am J Transplant. 2002; 2:25–30. [PubMed: 12095052]
19. Lopes da Silva R, Ferreira I, Teixeira G, Cordeiro D, Mafra M, Costa I, et al. Bk virus encephalitis with thrombotic microangiopathy in an allogeneic hematopoietic stem cell transplant recipient. Transpl Infect Dis. 13:161–7. [PubMed: 21054716]
20. Loechelt BJ, Boulware D, Green M, Baden LR, Gottlieb P, Krause-Steinrauf H, et al. Epstein barr and other herpes virus infections in early onset type i diabetics treated with daclizumab and mycophenolate mofetil. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2012

21. Pescovitz MD, Torgerson TR, Ochs HD, Ocheltree E, McGee P, Krause-Steinrauf H, et al. Effect of rituximab on human in vivo antibody immune responses. J Allergy Clin Immunol. 128:1295–302. e5. [PubMed: 21908031]

Appendix

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TABLE 1

PCR Primers and Probes

Analyte	Primer 1	Primer 2	Probe-FL	LC640-Probe-PH
CMV	ggcagctatcgtgactgg	gatccgacccattgtctaag	cgacggtgattcgtggtcgt	Ccaactggtgctgccggtcg
EBV	gagggtggtttggaaagc	aacagacaatggactcccttag	agtcgtctccccttggaatggc	ctggacccggcccacaacctg
JCV	aactaacatttcttctctggtc	actttcaggaaaacccac	gatgctgtcaacccttgtttgg	gctacagtatcaacagcctgct
BKV	acagcaaagcaggcaag	ggtgccaacctatggaacag	ttttgccatgaagaaatgtttgccagtgatga	aagcaacagcagattctcaacactcaaca

TABLE 2

Characteristics of the Study Groups

	Rituximab	Placebo
	N = 57	N = 30
Age — yr		
Mean ± standard deviation	19.0±8.6	17.3±7.8
Median	16	14
Range	8–40	9–38
Male sex : no. of patients (%)	36 (63)	18 (60)
Race or ethnic group : no. of patients (%)		
White	55 (96)	29 (97)
Non-Hispanic	54 (95)	27 (90)
No. of days from diagnosis to first infusion		
Median	81	91
Range	37–137	34–109

TABLE 3

Incidence of Primary Infections and Reactivations/Reinfections in Subjects at Risk

	EBV		CMV		BKV		JCV	
	Ritux	***Plac***	***Ritux***	***Plac***	***Ritux***	***Plac***	***Ritux***	***Plac***
N Baseline Seropositive/N Total*	27/51 (53%)	12/27 (44%)	16/51 (31%)	4/27 (15%)	43/52 (83%)	21/30 (70%)	16/52 (31%)	4/30 (13%)
N with Seroconversion/N Susceptible	0/24	0/15	0/35	0/23	0/30	1/9 (11%)	6/36 (17%)	2/26 (8%)
N with PCR+ Primary Infection/N Susceptible	0/24	0/15	0/35	0/23	0/30	0/9	1/36 (3%)	0/26
N Serologic Reactivation/N Baseline Seropositive	0/27	0/12	2/16 (13%)	1/4 (25%)	5/43 (12%)	2/21 (10%)	0/16	0/4
N PCR+ Reactivation/N Baseline Seropositive	**0/27**	**3/12 (25%)**	0/16	0/4	**4/43 (9%)**	**0/21**	0/16	0/4

Abbreviations: Ritux=rituximab; Plac=placebo; N=number

Bold font indicates significant differences ($p<0.05$).