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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.
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.

1. 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]

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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	gatgctgtcaaccctttgtttgg	gctacagtatcaacagcctgct
BKV	acagcaaagcaggcaag	ggtgccaacctatggaacag	ttttgccatgaagaaatgtttgccagtgatga	aagcaacagcagattctcaacactcaaca

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]

2. 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.

3. Study design

3.1. Subjects

Of 87 participants between 8 and 40 years old, 57 were randomly assigned to receive rituximab (Table 2). Four 375 mg/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.

3.2. 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 microliters 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 $MgCl_2$ (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 200 μl serum using the MagNA Pure apparatus Roche. Five microliters of extracted DNA were added to 15 μl of DNA PCR master mix containing LightCycler FastStart DNA reaction mix (Roche), $MgCl_2$ (3 mM for BKV and 4 mM for JCV), primers (0.5 mM each) and probes (0.25 mM 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.

3.3. 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 2 h at room temperature with 300 μl 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 1 h 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 405 nm in an automated microtiter plate reader (Molecular Devices) with a reference wavelength of 490 nm. A cut off value for seropositivity was defined as the optical density >mean + 3 S.D. of the reactivity

Table 2
Characteristics of the study groups.

	Rituximab *N* = 57	Placebo *N* = 30
Age – year		
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