Epstein-Barr and Other Herpesvirus Infections in Patients With Early Onset Type 1 Diabetes Treated With Daclizumab and Mycophenolate Mofetil

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(See the Editorial Commentary by Kumar and Humar, on pages 255–7.)

Background. We assessed the morbidity of herpesviruses in patients with type 1 diabetes mellitus (T1D) enrolled in immunosuppressive treatment studies.

Methods. Epstein-Barr virus (EBV), cytomegalovirus (CMV), herpes simplex virus (HSV), and varicella zoster virus (VZV) infections were monitored in 126 participants of a randomized, double-blind, placebo-controlled study of daclizumab (DZB) and mycophenolate mofetil (MMF) including DZB+MMF+, DZB−MMF+, DZB+MMF−, and DZB−MMF−. During the 2-year follow-up, herpesviral infections were monitored clinically, by serology and blood DNA polymerase chain reaction.

Results. Among 57 baseline EBV-seronegative participants, 9 developed EBV primary infections, including 2 with infectious mononucleosis syndrome. There were no appreciable differences in the course of the primary EBV infections across treatment groups. Among 69 baseline EBV-seropositive participants, 22 had virologic reactivations, including 1 symptomatic DZB−MMF+ subject. Compared with 7 DZB−MMF+ EBV reactivators, the 9 DZB+MMF+ reactivators tended to have more prolonged viremia (11.4 vs 4.4 months; \( P = .06 \)) and higher cumulative viral burden (14.2 vs 12.5 log EBV copies/mL; \( P = .06 \)). Four of 85 baseline CMV-seronegative subjects developed asymptomatic primary CMV infections. There were no CMV reactivations. Of 30 baseline HSV-seropositive subjects, 8 developed ≥1 episode of herpes labialis; 1 subject had a primary HSV infection; and 1 subject without baseline serology information had a new diagnosis of genital HSV. There were no significant differences in the incidence of HSV recurrences across treatment groups. Of 100 baseline VZV-seropositive subjects, 1 DZB−MMF− subject developed herpes zoster and 1 DZB−MMF+ subject had Bell’s palsy possibly related to VZV.

Conclusions. The use of DZB alone or in combination with MMF was not associated with increased morbidity due to herpesviruses.

Clinical Trials Registration. NCT00100178.

Keywords. type 1 diabetes; daclizumab; mycophenolate mofetil; Epstein-Barr virus; herpes viruses.

The use of immunosuppressive therapy for the treatment of autoimmune diseases is rapidly increasing. This approach is widely utilized for rheumatoid arthritis, systemic lupus erythematosus, and inflammatory bowel disease, but it is only in the experimental stage for the treatment of type 1 diabetes mellitus (T1D). The efficacy of cyclosporine A, anti-CD3, and anti-CD20 monoclonal antibodies to prevent, halt, or slow
progression of T1D have been investigated [1–3]. Although these interventions have not achieved a long-lasting therapeutic effect, they have shown temporary preservation of pancreatic β-cell function. Accordingly, there are ongoing efforts to evaluate additional agents in order to identify regimens that might result in long-lasting benefits. In this light, we recently completed a prospective, randomized, multicenter clinical trial evaluating the efficacy of mycophenolate mofetil (MMF) with or without daclizumab (DZB) as treatment for new-onset T1D [4].

The use of immunosuppressive agents raises concerns for potential infectious complications [5–7]. A particular focus of this concern is the impact on herpesviral infections. The herpesviridae family is characterized by life-long infection through the establishment of latency. Notorious pathogens are Epstein-Barr virus (EBV), cytomegalovirus (CMV), herpes simplex virus 1 and 2 (HSV1 and HSV2), and varicella zoster virus (VZV). Infection due to these viruses (primary or reactivation/reinfection) has been associated with increased morbidity and potentially mortality in immunosuppressed patients [8–18]. EBV infection in particular has been associated with the development of lymphoproliferative disease including lymphoma [6, 12, 19–25]. Accordingly, monitoring acquisition and reactivation of herpesviruses provides important safety data when evaluating potential immunosuppressive regimens.

This report reviews results of prospective serologic, virologic, and clinical monitoring for infection and disease due to herpesvirus infections in patients with new-onset T1D participating in a trial of MMF and DZB [4].

SUBJECTS AND METHODS

**Study Description**

The was a 4-arm, randomized, double-masked, placebo-controlled clinical trial conducted by the Type 1 Diabetes TrialNet at 13 sites in subjects aged 8–45 years with recognized T1D for <3 months [4]. The protocol was approved by the data and safety monitoring board (DSMB) and local institutional review boards. All subjects or caretakers provided written informed consent and/or assent.

Subjects were randomized to receive MMF with DZB (both from Roche Pharmaceuticals; DZB’MMF”), MMF and DZB-placebo (DZB”MMF”), MMF-placebo and DZB (DZB”MMF”), or MMF-placebo and DZB-placebo (DZB”MMF”).

MMF or placebo was administered daily at a dose of 600 mg/m² (maximum 2000 mg/day) in 2–3 doses for 2 years. DZB or placebo was given by intravenous infusion at study day 0 and 2 weeks later at a dose of 1 mg/kg. All subjects were followed for ≥2 years. Per protocol, study drugs were suspended in subjects who developed clinical or laboratory evidence of EBV or CMV primary infections or reactivations.

**Laboratory Studies**

Subjects had CMV, EBV, HSV, and VZV serologic evaluations and EBV and CMV polymerase chain reaction (PCR) at baseline. CMV and EBV infections were monitored weekly by serology and PCR during the first 4 weeks of study and biweekly during the subsequent 4 weeks. Thereafter, EBV- and CMV-seronegative subjects were followed monthly, and EBV- and CMV-seropositive subjects were followed at 3-month intervals. Per protocol, if EBV or CMV viremia developed, the PCR was repeated weekly during the first 4 weeks, biweekly during the subsequent 4 weeks, and monthly thereafter until it resolved.

**Real-Time Quantitative EBV DNA PCR**

A validated quantitative EBV DNA PCR in whole blood was performed as previously described [26]. The lower limit of detection was 100 copies/mL and the dynamic range was ≥500 DNA copies/mL.

**Real-Time Quantitative CMV DNA PCR**

Real-time quantitative CMV DNA PCR was performed following the same protocol described for EBV DNA PCR, but with the following primers and probes from Eco R1 region D: GGCAAGCTATCGTAGCTGG and GATCCGACCCATTTGCT TAAG and probes CGACGGTATTGCTGTGGT-fluorescein and LC Red640 –CCCACTGTGCTGGCGGTCG-phosphate elongation block. The analytical sensitivity was ≥100 DNA copies/mL and dynamic range ≥500 copies/mL.

**CMV, EBV, HSV, and VZV Serology**

Antibodies were measured using the following Food and Drug Administration–approved kits: DiamedixIs-CMV immunoglobulin G (IgG) Test Kit (Diamedix Corp), Premier CMV immunoglobulin M (IgM) enzyme-linked immunosorbent assay (ELISA) System (Meridian Bioscience), Premier EBV viral capsid antigen (VCA) IgG ELISA System (Meridian Bioscience), EBNA1 (Epstein-Barr nucleo antigen 1) IgG II (Wampole), EBV VCA IgG II (Wampole), and MERIFLUOR EBV IgM immunofluorescent assay (Meridian Bioscience). HSV and VZV IgG were measured using Diamedix HSV 1&2 IgG and VZV IgG, respectively (Diamedix). All assays were performed as per the manufacturers’ instructions.

**Clinical Data Collection and Management**

New signs and symptoms were spontaneously reported by the subjects or guardians, but also solicited at each study visit for the interval between visits. Intercurrent illnesses were reported through the adverse event reporting system. Each event was investigated by the clinical site personnel as per local standard of care or in consultation with the T1D TrialNet Infectious Diseases group. In addition, if a subject developed CMV or EBV viremia confirmed by 2 sequential positive PCR results,
treatment was suspended until the viremia disappeared as defined by 2 sequential negative PCR results.

All adverse events were reviewed by the medical monitor in a treatment-masked fashion and classified according to the Common Terminology Criteria for Adverse Events version 3 (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm). The DSMB received quarterly summary safety reports.

Statistical Analysis

Descriptive statistics, $t$ test (or the Wilcoxon rank sum test), and Fisher exact test were used to analyze the characteristics of the 4 cohorts. The Anderson-Darling statistic was used to assess normality; where appropriate, data were log transformed prior to testing. The area under the concentration-time curve (AUC) was calculated using the trapezoidal rule. For seroconverters who did not have a detectable EBV load (<100 copies/mL), a value of 99 was assigned for the calculation of the AUC. SAS G2 software was used in all analyses.

RESULTS

Demographic and Other Characteristics of the Study Population

The 126 subjects in the study had similar demographic characteristics across the 4 treatment arms (Table 1), including median age of 15 years (range, 9–46 years); 60% male sex; and 93% white race. At baseline, 55% were seropositive for EBV, 32% for CMV, 19% for HSV, and 84% for VZV. All subjects received the DZB or placebo infusions. The mean duration of MMF or treatment placebo was 20 months (SD, 6 months) months and the mean duration of follow-up on study was 23 months (SD, 4 months). The treatment did not have a significant impact on the progression of T1D.

EBV Primary Infections and Reactivations

There were 8 reports of mononucleosis-like syndromes. Two occurred in subjects with primary EBV infection and one in a subject who was experiencing an EBV reactivation. The remaining 5 subjects did not have any evidence of an associated active EBV infection by serology or PCR. All clinical symptoms in subjects with EBV-associated mono-like syndromes resolved without therapeutic interventions. However, study medication was withheld per protocol as noted above.

Primary infections were documented by positive EBV PCR in 9 of 57 subjects who were EBV-seronegative at baseline: 3 each in the DZB+MMF+ and DZB+MMF− groups, 1 in the DZB−MMF+ group, and 2 in the DZB−MMF− group (Table 2). The magnitude of the viremia measured by AUC varied from 12.3 to 16.3 log_{10} EBV DNA copies/mL and did not appear grossly different among treatment groups. Eight of the 9 subjects had ≥1 serum sample tested for EBV-specific antibodies after the positive PCR result. Of the 8 subjects with
serologic follow-up, all seroconverted for IgG anti-VCA at an average of 6.2 weeks after the first positive PCR result. Six and 7 subjects also developed positive IgM anti-VCA and IgG anti-EBNA, respectively, at an average of 4.7 and 15.7 weeks, respectively, after the first positive PCR result. The subject who did not develop anti-EBNA antibodies had the last serologic evaluation at the same visit when the first positive EBV blood PCR was detected. Formal statistical comparisons were not performed across the treatment groups owing to the small number of primary EBV infections in each group. Two subjects with primary EBV infection had symptomatic disease that manifested as infectious mononucleosis syndrome. Both clinical disease and viremia resolved without use of antiviral therapy.

Among 69 subjects with serologic evidence of prior EBV infection, 22 had reactivations detected by EBV PCR in whole blood. The 22 EBV reactivators had an average of 0.9 EBV PCR tests per month prior to the first positive PCR result, which was similar to the overall frequency of EBV PCR tests performed in the nonreactivators (1 PCR per month). Reactivations occurred in 9 DZB+MMF+, 2 DZB+MMF−, 4 DZB−MMF+, and 7 DZB−MMF− subjects (Table 3). The average time from study entry to the time when the first positive PCR was observed was 11.4 months for DZB+MMF+, 5.6 for DZB+MMF−, 6.3 for DZB−MMF+, and 4.4 for DZB−MMF−. At the time of the reactivation, 8 subjects were receiving active drug. Of the remaining 14, 9 were on placebo and 5 had already completed or interrupted treatment and were being followed off medication. In subjects still on treatment (active or placebo), the study medication was stopped after 2 consecutive positive EBV PCR results. Nevertheless,

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DZB+ MMF+</th>
<th>DZB+ MMF−</th>
<th>DZB− MMF+</th>
<th>DZB− MMF−</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. positive/total (%)</td>
<td>9/26 (35)</td>
<td>2/7 (29)</td>
<td>4/15 (27)</td>
<td>7/21 (33)</td>
<td>22/69 (32)</td>
</tr>
<tr>
<td>Age, years, median (range)</td>
<td>19.2 (10.4–40.3)</td>
<td>39.0 (36.0–42.1)</td>
<td>23.2 (9.8–24.4)</td>
<td>14.9 (11.9–44.5)</td>
<td>20.3 (9.8–44.5)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male, No. (%)</td>
<td>3 (33.3)</td>
<td>2 (100)</td>
<td>1 (25.0)</td>
<td>6 (85.7)</td>
<td>12 (55)</td>
</tr>
<tr>
<td>Female, No. (%)</td>
<td>6 (66.7)</td>
<td>...</td>
<td>3 (75.0)</td>
<td>1 (14.3)</td>
<td>10 (45)</td>
</tr>
<tr>
<td>Months on study before reactivation, mean (SD)</td>
<td>14.3 (8.5)</td>
<td>3.0 (0.6)</td>
<td>19.0 (12.6)</td>
<td>5.1 (3.2)</td>
<td>11.2 (9.4)</td>
</tr>
<tr>
<td>Months of EBV viremia, mean (SD)</td>
<td>11.4 (11.4)</td>
<td>5.6 (8.0)</td>
<td>6.3 (11.5)</td>
<td>4.4 (9.4)</td>
<td>7.7 (10.4)</td>
</tr>
<tr>
<td>Peak log EBV DNA, copies/mL, mean (SD)</td>
<td>9.2 (2.2)</td>
<td>7.8 (0.6)</td>
<td>8.6 (3.2)</td>
<td>7.9 (1.9)</td>
<td>8.5 (2.2)</td>
</tr>
<tr>
<td>AUC log EBV DNA, copies/mL, mean (SD)</td>
<td>14.2 (2.0)</td>
<td>12.0 (0.2)</td>
<td>13.4 (2.7)</td>
<td>12.5 (1.5)</td>
<td>13.4 (2.0)</td>
</tr>
</tbody>
</table>

Abbreviations: AUC, area under the concentration-time curve; DZB, daclizumab; EBV, Epstein-Barr virus; MMF, mycophenolate mofetil; SD, standard deviation.

Table 2. Characteristics of Subjects With Primary Epstein-Barr Virus Infections

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DZB+ MMF+</th>
<th>DZB+ MMF−</th>
<th>DZB− MMF+</th>
<th>DZB− MMF−</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. primary/No. seronegative (%)</td>
<td>3/15 (20)</td>
<td>3/5 (60)</td>
<td>1/16 (8)</td>
<td>2/21 (10)</td>
<td>9/57 (16)</td>
</tr>
<tr>
<td>Age, years, median (range)</td>
<td>18.4 (14.5–32.0)</td>
<td>15.3 (11.7–18.2)</td>
<td>12.4</td>
<td>20.1 (16.3–23.9)</td>
<td>16.3 (11.7–32.0)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male, No. (%)</td>
<td>2 (66.7)</td>
<td>2 (66.7)</td>
<td>...</td>
<td>...</td>
<td>4 (44)</td>
</tr>
<tr>
<td>Female, No. (%)</td>
<td>1 (33.3)</td>
<td>1 (33.3)</td>
<td>1 (100)</td>
<td>2 (100)</td>
<td>5 (66)</td>
</tr>
<tr>
<td>PCR-positive, No.</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>AUC log EBV DNA/mL, mean (SD)</td>
<td>15.3 (1.2)</td>
<td>13.7 (1.4)</td>
<td>12.8 (…)</td>
<td>16.3 (0.05)</td>
<td>14.7 (1.6)</td>
</tr>
<tr>
<td>No. with IgM VCA seroconversion</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Months from first PCR to first positive IgM+, mean (SD)</td>
<td>0.5 (0.7)</td>
<td>0</td>
<td>0.2</td>
<td>2.6 (1.4)</td>
<td>1.1 (1.4)</td>
</tr>
<tr>
<td>No. with IgG VCA seroconversion</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Months from PCR to first positive IgG+, mean (SD)</td>
<td>1.7 (1.4)</td>
<td>0.4 (0.7)</td>
<td>0.2</td>
<td>2.6 (1.4)</td>
<td>1.4 (1.4)</td>
</tr>
<tr>
<td>No. (%) EBNA seroconversion</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>7</td>
</tr>
</tbody>
</table>

Abbreviations: AUC, area under the concentration-time curve; DZB, daclizumab; EBNA, Epstein-Barr nuclear antigen; EBV, Epstein-Barr virus; IgG, immunoglobulin G; IgM, immunoglobulin M; MMF, mycophenolate mofetil; PCR, polymerase chain reaction; SD, standard deviation; VCA, viral capsid antigen.

* Means were calculated based on data from seroconverters only. Serology was performed at monthly intervals.
DZB+MMF+ subjects tended to have more prolonged episodes of reactivation compared with placebo (11.4 vs 4.4 months; \(P = .06\)) and marginally higher AUC for viremia (14.2 vs 12.5 \(\log\) EBV DNA copies/mL; \(P = .06\)). The DZB+MMF+ and DZB+MMF− groups had too few subjects with reactivation to perform meaningful comparisons.

One of the subjects with EBV reactivation developed monolike symptoms 8 months after the first positive EBV PCR result. The symptoms resolved spontaneously, but the subject continued to be viremic for an additional 16 months, when study follow-up was discontinued. The subject was in the DZB+MMF− group.

**CMV Primary Infections and Reactivations**

At baseline, 39 of 124 participants (31.4%) were CMV-seropositive. Four subjects, 1 each in the DZB+MMF+ and DZB+MMF− groups and 2 in the DZB+MMF+ group, acquired primary CMV infection during the study documented by seroconversion. Seroconversions were confirmed by serology results at subsequent visits. All CMV primary infections were asymptomatic. Blood CMV PCR results were negative in all subjects with primary infections at and before seroconversion as measured through the monthly monitor. There were no positive CMV PCR results in CMV-seropositive subjects.

**HSV Primary Infections and Reactivations**

At baseline, 30 of 107 participants with available serum samples (28%) were HSV-seropositive and 4 subjects seroconverted during the study. Ten subjects developed symptomatic HSV infections, consisting of 1 genital and 15 orolabial episodes (including 4 subjects with 2–3 recurrences each). Four subjects with symptomatic HSV were in the DZB+MMF+ group, 4 in the DZB+MMF− group, and 2 in the DZB+MMF+ group. One of the DZB+MMF− subjects had a primary HSV gingivitis and 1 subject in the DZB+MMF+ who had a genital HSV episode diagnosed for the first time during the study did not have baseline serology data. The remaining 8 subjects had recurrent orolabial HSV. The incidence of recurrent HSV infections was not significantly different among the 4 treatment groups (\(P = .25\), Fisher exact test). Four subjects received antiviral therapy including 2 subjects who also were prescribed prophylactic antivirals due to multiple recurrences. The other episodes resolved without antiviral treatment. One subject with multiple recurrences also stopped study medication during one of the HSV recurrences.

**VZV Reactivations**

Of 121 subjects, 102 (84%) were VZV-seropositive at baseline. There was an episode of herpes zoster in this study documented in a DZB+MMF− recipient. The subject had a self-limited rash that resolved after 7 days of therapy with valacyclovir and was not complicated by postherpetic neuralgia. A VZV-seropositive, HSV-seronegative subject in the DZB+MMF− group experienced an episode of Bell’s palsy, which is deemed to be sometimes due to HSV or VZV reactivation [27, 28]. The subject was treated with valacyclovir by his primary care physician without any specific evidence of VZV reactivation. Clinical symptoms improved on therapy.

**DISCUSSION**

We did not find any significant morbidity attributable to herpesviruses in individuals with early onset T1D treated with MMF and/or DZB despite having active surveillance in place for the potential development of these infections. This finding was reassuring in view of the rapidly growing use of immunosuppressive regimens to treat and/or prevent inflammatory and autoimmune disorders including T1D. However, our sample size was relatively small. It is also important to note that the combination of MMF and DZB did not have a significant effect on the progression of diabetes [4], while limited success has been reported with other immunosuppressive regimens [1–3, 29]. One of the potential explanations for the therapeutic failure of MMF/DZB is that this regimen is not potent enough to modulate the immune response against the pancreatic islet cells or did not target the mechanisms relevant for T1D. The corollary is that more potent regimens may be associated with a higher incidence of opportunistic infections.

EBV primary infections and reactivations were closely monitored due to the oncogenic potential of this virus and the established role of immunosuppression on EBV-associated morbidity and mortality in other populations. The 9 EBV primary infections (2 symptomatic) detected in this study were roughly equally distributed across treatment groups. Although the numbers were too small for a formal statistical analysis, there were no gross differences in the course of the primary infections across treatment groups. All subjects with serologic follow-up after the primary EBV infection developed IgG anti-VCA antibodies, suggesting that the ability to mount humoral immune responses was not grossly affected by any of the drug combinations. IgM responses were demonstrated in 75% of the subjects with serologic follow-up, which is in agreement with the frequency of IgM responses in immunocompetent individuals with EBV-associated infectious mononucleosis [30, 31]. All subjects with ≥1 month of follow-up after the first positive PCR result developed anti-EBNA antibodies. Lack of seroconversion to EBNA has been associated with increased risk of posttransplant lymphoproliferative disorder in the transplant population [12, 15, 16, 32]. Hence, it was reassuring to document EBNA seroconversion in all the
subjects with primary EBV infection who had an adequate length of follow-up in this study.

EBV reactivations occurred in 30% of the EBV-seropositive subjects; the rate of reactivation was similar across treatment groups. Asymptomatic EBV reactivations have been frequently described in healthy blood donor and other immunocompetent individuals [13, 33]. However, the studies in healthy volunteers provided cross-sectional information on EBV reactivation, whereas our data were longitudinal, making it difficult to compare the rates of EBV reactivation in our subjects with T1D with those reported in the general population. It is noteworthy that a rate of EBV reactivation similar to the one in this study was found in T1D placebo recipients enrolled in other TrialNet intervention studies (J. Kroll et al, manuscript in preparation). The viral loads during reactivations could vary in magnitude by >1 log10 between sequential visits, but overall they did not appreciably differ from EBV viral loads measured in transplant recipients in the same laboratory [26]. The duration of reactivations was also highly variable from a single visit (confirmed with a second test within the following week) to >24 months. Only 1 subject had symptoms consistent with infectious mononucleosis during an EBV reactivation. However, because the reactivation started 8 months before the subject became symptomatic and extended for another 16 months after symptoms resolved, it is unclear whether EBV was truly responsible for this adverse event. Previous studies using anti-CD3 humanized mouse monoclonal antibodies to prevent progression of early onset T1D showed infectious mononucleosis-like symptoms in 75% of the active treatment recipients [1]. However, in our study, perhaps due to the lower intensity of the immunosuppression, EBV reactivations were not associated with significant clinical consequences regardless of treatment group.

Although there were no clinical differences among treatment arms, the DZB+MMF subjects had protracted viremia and higher copy numbers of circulating EBV DNA compared with the DZB MMF controls. This suggests that the 2-drug combination had an immunologic effect, albeit modest.

There were no CMV reactivations documented by CMV DNA PCR in whole blood. There were 4 CMV primary infections during the study, all of which were asymptomatic and were not accompanied by viremia. Although it is possible that brief transient episodes of viremia occurred between scheduled assays, overall, our findings indicate that the immunosuppression in the MMF and/or DZB recipients was not intensive enough to cause CMV-associated morbidity.

Herpes zoster is a common complication of cell-mediated immune suppression. However, in this study, the incidence of herpes zoster was in line with the 0.15 per 100 person-years incidence of herpes zoster reported in the age-matched general population (10–19 years) [34]. Taken together, the data indicate that the rate of symptomatic VZV reactivations was not adversely affected by treatment or T1D in this small study.

HSV reactivations seemed to be clustered in the DZB+MMF and DZB+MMF arms, but the differences did not reach statistical significance. Furthermore, reactivation episodes that were not treated with antivirals showed normal healing and episodes of primary or first nonprimary HSV infections subsided after 3–5 days of antiviral therapy. The data indicate that although HSV symptomatic infections were not uncommon in this population, their morbidity did not appear to differ from that of HSV disease in immunocompetent individuals.

The MMF/DZB study was the first intervention trial using immunosuppressive therapy conducted by the T1D TrialNet Study Group. In the absence of extensive data regarding the clinical course of herpesvirus infections in T1D patients treated with immunosuppressive drugs, it was deemed important to closely monitor these infections, particularly EBV and CMV. Retrospectively, the MMF/DZB combination was safe and the cost of the monitor was higher than warranted. Nevertheless, this study provided new data on the natural history of herpesvirus infections in T1D individuals with or without MMF/DZB treatment, including the high frequency of EBV reactivations in double-placebo recipients, that will be useful in planning viral monitors in future studies.

**Notes**

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