Human Herpesvirus 6 Reactivation and Encephalitis in Allogeneic Bone Marrow Transplant Recipients

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To determine whether receipt of an investigational anti-CD3 monoclonal antibody (BC3) increased the risk of human herpesvirus 6 (HHV-6) reactivation and development of encephalitis in bone marrow transplant (BMT) recipients, persons who had and had not received BC3 were compared. Odds of HHV-6 reactivation were higher among BC3 recipients than among control patients (odds ratio, 2.5; 95% confidence interval [CI], 1.3–4.7). In addition, BC3 recipients were more likely than control patients to develop encephalitis (risk ratio [RR], 3.5; 95% CI, 1.3–9.5), and this association followed a BC3 dose–dependent relationship (P = .03, by Mantel-Haenszel χ² test). In a multivariable model, HHV-6 reactivation and receipt of BC3 were associated with increased risk of encephalitis (RR, 5.4; 95% CI, 1.9–15.3, and RR, 3.3; 95% CI, 1.2–9.1, respectively). In conclusion, both HHV-6 reactivation and receipt of BC3 for prophylaxis of acute graft-versus-host disease independently increased the risk of encephalitis in allogeneic BMT recipients. Prospective studies to better define the relationship between HHV-6 reactivation and encephalitis in allogeneic BMT recipients are warranted.

Human herpesvirus 6 (HHV-6) is an ubiquitous virus that infects most persons by 2 years of age [1–3]. Primary infection occurs in healthy children and commonly causes fever and other self-limited symptoms [4]. The course is benign in the majority of persons, but cases of more severe primary infection complicated by encephalitis have been reported [5–11]. After primary infection, the virus persists in lymphocytes and salivary glands. It is unknown to what extent the virus reactivates in healthy adults and what, if any, clinical significance might be associated with reactivation. Immunocompromised persons, such as bone marrow transplant (BMT) recipients, can have an increase in HHV-6 replication in blood [12–14], but the relationship between viral replication and clinically recognizable disease has been difficult to establish. HHV-6 has been postulated to be a cause of encephalitis among BMT recipients, on the basis of the presence of HHV-6 in the CSF or brain tissue and consistent clinical signs and symptoms [15–21].

In the course of conducting phase I/II studies of BC3 [22, 23], an anti-CD3 monoclonal antibody evaluated for prophylaxis of acute graft-versus-host disease (GVHD) in allogeneic BMT recipients, 2 patients developed encephalitis accompanied by high levels of HHV-6 DNA in their CSF. This observation led us to conduct a retrospective cohort study to evaluate whether receipt of BC3 was associated with encephalitis and HHV-6 reactivation in allogeneic BMT recipients.
METHODS

Subjects
Medical records from the period 1985–1998 were reviewed at the Fred Hutchinson Cancer Research Center (Seattle) to identify recipients of first-time allogeneic BMTs who had received BC3 for prophylaxis of acute GVHD (n = 51). These patients were enrolled in 1 of 2 consecutive phase 1 dose-escalation studies of BC3 administered with or without glucocorticoids. The first trial included 40 recipients of related donor transplants with identical human leukocyte antigen (HLA) haplotypes that were incompatible for 2 or 3 HLA-A, -B, and -DR antigens of the nonshared haplotype. The second trial included 11 recipients of unrelated donor transplants compatible for HLA-A, -B, and -DR. Bone marrow cells were infused without T cell depletion. The day of bone marrow transplantation was designated day 0. Conditioning included cyclophosphamide administered iv at 60 mg/kg on 2 consecutive days, followed by total body irradiation with 120 cGy, 11 fractions over the course of 4 days for patients ages ≥18 years or 12 fractions over the course of 4 days for patients <18 years. BC3 was administered iv on day −1 at a loading dose that varied among patients from 0.2 to 3.2 mg/kg (median, 0.4 mg/kg) and on days 0–12 or 0–20 at a maintenance dose of 0.1–1.6 mg/kg/day (median, 0.2 mg/kg/day). Methylprednisolone was administered iv to 32 (62%) of 51 BC3 recipients at a dose of 2 mg/kg/day on days −1 to 12, followed by a slow tapering over the course of 50 days. Oral prednisone could be substituted at an equivalent dose by use of a 5:4 ratio. Cyclosporine was given at the standard dose of 1.5 mg/kg iv q12h and was started on day −1, for patients who did not receive glucocorticoids, or on day 12 or 20 after administration of BC3, for patients who did receive glucocorticoids. Oral cyclosporine could be substituted for the iv formulation. Methotrexate was administered to the 40 recipients of HLA haplotype–identical related donor transplants. Dosing of methotrexate started 24 h after completion of the bone marrow infusion and was given at 15 mg/m² for the first dose and 10 mg/m² for subsequent doses (2 days, 5 days, and 10 days after the first methotrexate dose). Treatment protocols for acute GVHD consisted of early administration of cyclosporine, in-
TGA CTG GCA AAA. The EBV primers EBER1-A and EBER1-B were used to amplify a 118-bp region of EBER1 [27], and the CMV primers Cyto969F (5′-CCA GTG CCC GCA GTT TTT ATT) and Cyto1054R (5′-ACC GGA GAA GAG CCC ATG TC) were used to amplify an 86-bp region of the immediate-early protein gene. The dual-labeled fluorescent EBER1-P-TAQ probe (5′-CCA CAG ACA CCG TCC TCA CCA CCC G) and CytoG probe (5′-AAC ATA ACG TGG GAT CTC CAC GCG AAT) were designed for the real-time PCR fluorescent dye system to detect EBER1-A/B and Cyto969F/1054R PCR products, respectively, as described elsewhere for HHV-6 [28].

PCR was done and analyzed by use of a Perkin-Elmer Applied Biosystems Sequence Detector 7700, as described elsewhere [28], with the exception that 0.275 μg (0.25 μL) of TaqStart antibody (Clontech) and 0.05 U of uracil DNA glycosylase (EpiCentre Technology) were included in all reactions, and 400 nM of Cyto969F/1054R primers was included when CMV DNA was amplified. The limit of detection of the assays was 1 copy of viral DNAs per reaction (20 μL of serum assayed) or 50 copies/mL of serum. In all reactions, the EXO noncompetitive internal control was used instead of the Fly-C internal control to ensure that negative results were not due to nonspecific inhibition of the PCR. To accomplish this, each PCR reaction was spiked with 50 copies of internal control DNA (EXO), 30 nM of primers (EXO186F and EXO314R), and 50 nM of probe (PMP-242T). The EXO DNA sequence is GCC TGG TGC CAA AAA TTG CTT ATT ATC AAT TGA ACG GTC AAT TGG TGG AAG TGG CGG AAG AAC AGC TAT TGC AAA CGC CAT GCG ACA ATA CCA TAA ACA CAC TTG TCT TAG GTT CAC AAA AGA ACA AAT GAA CGA. The EXO186F and EXO314R sequences are GCC TGG TGC CAA AAA ATG TGC TT and TCG TCC ATT TGT TCT TCT TGT GTG GAA, respectively. PMP-242T (CAG CTA TTG CAA ACG CCA TCG CAC) is labeled at the 5′ end with VIC (Perkin-Elmer) and at the 3′ end with 6-carboxytetramethylrhodamine. All of the negative HHV-6 PCR results required detection of EXO DNA.

Analysis

Statistical analysis was done with SPSS (SPSS, Inc.) and SAS (SAS Institute) software programs. Proportions were compared between groups with the χ² test or, if assumptions required for the χ² test were not met, with Fisher’s exact test. Continuous variables were compared between groups by the 2-sample t test or, if assumptions necessary for the t test were not met, the Wilcoxon rank sum test. Whether a dose-response relationship existed between BC3 and encephalitis was evaluated by means of Mantel-Haenszel χ² test. The probability of encephalitis was summarized with a cumulative incidence estimate [29], in which death without encephalitis was regarded as a competing risk. The odds of virus reactivation were assessed by means of generalized estimating equations [30] with a logistic link func-

RESULTS

Index Case

A 51-year-old man received an HLA-matched unrelated donor BMT for chronic-phase myelogenous leukemia. His conditioning regimen included cyclophosphamide and total body irradiation (12 Gy), and he received BC3 (0.4 mg/kg loading dose on day −1, followed by 0.2 mg/kg/day from day 0 through day 20) and methylprednisolone (2 mg/kg/day from day −1 through day 12, followed by a tapering dose) for GVHD prophylaxis. On day 14 after transplantation, he developed headaches and high urine output associated with sodium chloride wasting. On day 15, he developed fever, followed by confusion, profound recent memory loss, lethargy, and horizontal nystagmus. A head MRI on day 19 yielded normal results. CSF obtained by lumbar puncture on day 20 revealed normal levels of glucose and protein and 4 WBCs (peripheral WBC count at this time was 50,000/μL of serum); absolute neutrophil count was 730 cells/mm³. PCR of CSF for herpes simplex virus, varicella-zoster virus, and CMV yielded negative results, but PCR for HHV-6 revealed >50,000 copies of HHV-6 DNA per mL. He was treated with ganciclovir and foscarnet, and his condition improved, but short-term memory loss persisted.

Cohort Study

Demographic and clinical characteristics. All persons who received BC3 for prophylaxis of acute GVHD after allogeneic BMT (51 patients) at some point from 1985 through 1998 were compared with a similar group of allogeneic BMT recipients who did not receive BC3 (103 patients) from the same time period. Demographic and clinical characteristics were similar between BC3 recipients and control patients, except that BC3 recipients were more likely to be nonwhite, to receive a lower dose of total body irradiation, and to develop higher-grade acute GVHD than control patients (table 1). In addition, a greater
number of BC3 recipients than of control patients received glucocorticoids. Thirty-six (70%) of the 51 BC3 recipients received glucocorticoids at the time of transplantation as part of a BC3 protocol, and the remaining 15 (30%) received glucocorticoids after transplantation for treatment of acute GVHD.

Encephalitis. Encephalitis occurred more frequently in BC3 recipients than in control patients (13 [25.5%] of 51 vs. 13 [12.6%] of 103; \( P = .05 \)). The association between encephalitis and receipt of BC3 was dose related (\( P = .03 \), by Mantel-Haenszel \( \chi^2 \) test) (figure 1). All BC3 recipients who developed encephalitis were receiving glucocorticoids before that event. Among BC3 recipients, the frequency of encephalitis did not differ between those who received glucocorticoids at the time of transplantation (8 [22%] of 36) and those who received them later in the course of the transplantation (5 [33%] of 15; \( P = .49 \), by Fisher’s exact test). Evaluation of the 26 patients with encephalitis included lumbar puncture in 17 (65%), head MRI or CT in 23 (88%), and electroencephalography in 13 (50%). Results revealed that 5 (29%) of the 17 patients who underwent lumbar puncture had elevated CSF protein levels, 1 (6%) of the 17 had CSF pleocytosis, 2 (9%) of the 23 patients who underwent head imaging studies had abnormal results that were consistent with an acute process other than hemorrhage and infarction, and 9 (69%) of 13 patients who underwent encephalography had abnormal results.

Of the 13 BC3 recipients and 13 control patients with encephalitis, most also had seizure (11 [85%] and 10 [77%], respectively). There was a suggestion that seizures occurred more frequently in BC3 recipients (12 [23.5%] of 51 vs. 13 [12.6%] of 103; \( P = .12 \)).

HHV-6 reactivation. Routine CSF sampling was not part of the transplantation procedure or BC3 protocol. Therefore, we used stored sequential serum samples to define the time course and frequency of HHV-6 reactivation. HHV-6 and EBV reactivation occurred more frequently among BC3 recipients than among control patients, whereas there was no difference between the groups with regard to CMV reactivation (table 2). The frequency of HHV-6 reactivation was greatest from 2 through 5 weeks after transplantation, which corresponded to the time period when most cases of encephalitis occurred (median, 24.5 days after BMT) (figure 2). The proportion of persons who received acyclovir did not differ between BC3 recipi-

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control patients (n = 103)</th>
<th>BC3 recipients (n = 51)</th>
<th>( P^a )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, male</td>
<td>65 (63.5)</td>
<td>32 (62.7)</td>
<td>.97</td>
</tr>
<tr>
<td>Race, nonwhite</td>
<td>7 (6.8)</td>
<td>10 (19.6)</td>
<td>.02</td>
</tr>
<tr>
<td>Mean age, years (SD)</td>
<td>22.0 (15.0)</td>
<td>24.3 (17.8)</td>
<td>.41b</td>
</tr>
<tr>
<td>Primary diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute myelogenous leukemia</td>
<td>22 (21.4)</td>
<td>13 (25.5)</td>
<td>.29</td>
</tr>
<tr>
<td>Chronic myelogenous leukemia</td>
<td>41 (39.8)</td>
<td>16 (31.4)</td>
<td></td>
</tr>
<tr>
<td>Acute lymphocytic leukemia</td>
<td>27 (26.2)</td>
<td>19 (37.3)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>13 (12.6)</td>
<td>3 (5.9)</td>
<td></td>
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<tr>
<td>Unrelated donorc</td>
<td>32 (31.1)</td>
<td>11 (21.6)</td>
<td>.22</td>
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<tr>
<td>Whole brain irradiation</td>
<td>15 (14.6)</td>
<td>8 (15.7)</td>
<td>.86</td>
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<td>Pretransplant intrathecal chemotherapy</td>
<td>38 (36.9)</td>
<td>24 (47.1)</td>
<td>.23</td>
</tr>
<tr>
<td>Prior CNS leukemia</td>
<td>10 (9.7)</td>
<td>7 (13.7)</td>
<td>.45</td>
</tr>
<tr>
<td>Preparation with cyclophosphamide and TBI alone</td>
<td>96 (93.2)</td>
<td>51 (100)</td>
<td>.10</td>
</tr>
<tr>
<td>TBI dose (&lt;=1440) cGyd (^d)</td>
<td>47 (45.6)</td>
<td>46 (90.2)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Glucocorticoids</td>
<td>89(^e) (87)</td>
<td>51 (100)</td>
<td>.005</td>
</tr>
<tr>
<td>Antithymocyte globulin</td>
<td>22(^e) (22)</td>
<td>15 (29)</td>
<td>.29</td>
</tr>
<tr>
<td>Acute GVHD, grades III–IV</td>
<td>47 (45.6)</td>
<td>33(^f) (67.3)</td>
<td>.01</td>
</tr>
<tr>
<td>Median maximum bilirubin level, mg/dL (range)</td>
<td>5.9 (0.8–87.2)</td>
<td>8.3 (0.6–59)</td>
<td>.55g</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. (%) of patients, unless otherwise noted. GVHD, graft-versus-host disease; HLA, human leukocyte antigen; TBI, total body irradiation.

\( a \) By Pearson \( \chi^2 \), unless otherwise noted.

\( b \) By Student’s \( t \) test.

\( c \) Related donors had identical HLA haplotype and were mismatched for 2 or 3 HLA-A, -B, and -DR antigens.

\( d \) Dose of TBI was not greater than 1575 cGy for the remainder of the patients.

\( e \) Data were unavailable for 1 subject; n = 102.

\( f \) Data were unavailable for some subjects; n = 49.

\( g \) By Wilcoxon rank sum test.
patients and control patients (20 [56%] of 36 vs. 53 [62%] of 86; P = .53). Foscarnet and/or ganciclovir was used more frequently to treat BC3 recipients than to treat control patients (15 [42%] of 36 and 19 [22%] of 86, respectively; P = .03). Only 1 subject received foscarnet or ganciclovir before the development of encephalitis, and this person did not have evidence of HHV-6 reactivation.

The quantity of HHV-6 viremia over time was higher in those who developed encephalitis than in those who did not (figure 3). Patients with levels of HHV-6 DNA >5000 copies/mL appeared more likely to develop encephalitis than did those with lower levels: 7 (24%) of 29 versus 11 (12%) of 94. In all, 13 BC3 recipients and 13 control patients developed encephalitis. Of the BC3 recipients with encephalitis, 9 had serum specimens available for HHV-6 testing that were obtained before the patient developed encephalitis, 1 only had serum specimens that were obtained after the onset of encephalitis, and 2 did not have serum specimens available. Of the 9 BC3 recipients who had serum specimens available that were obtained before encephalitis developed, 7 (78%) had HHV-6 DNA isolated. One of the patients who had a negative result on testing of serum obtained before the onset of encephalitis had a positive result 2 days after the diagnosis of encephalitis and also had HHV-6 DNA isolated from CSF (index case). The patient who only had serum specimens available that were obtained after encephalitis developed had a specimen from 7 days after the onset of encephalitis that tested positive for HHV-6 at 215,000 copies of DNA per mL. In all, 9 (90%) of 10 BC3 recipients with encephalitis who had any serum specimens available for testing had HHV-6 isolated from their serum. Of the 13 control patients who developed encephalitis, 7 had serum specimens that were obtained before encephalitis developed, and 1 had a serum specimen available for HHV-6 testing that was obtained after encephalitis developed. Of the 7 who had serum specimens available for testing that were obtained before encephalitis developed, 3 (42.9%) had HHV-6 DNA isolated. One control patient for whom initial results of testing were negative had a positive result of testing of a serum specimen 2 days after the onset of the encephalitis, and 1 patient who did not have serum specimens available that were obtained before encephalitis developed had a positive specimen 12 days after the onset of encephalitis. In all, 5 (63%) of 8 control patients with encephalitis who had any serum specimens available for testing had HHV-6 isolated at approximately the time that encephalitis developed.

**Multivariable analysis.** Cox multivariable regression revealed that BC3 recipients had an increased risk of developing encephalitis compared with control patients, and this association was not altered after adjustment for virus reactivation (table 3). After adjustment for receipt of BC3, however, HHV-6 reactivation was associated with an increased risk of encephalitis. Other covariates, including donor type (unrelated vs. related), age of subject, race of subject, occurrence of acute high-grade GVHD, receipt of glucocorticoids, receipt of antithymocyte globulin, previous intrathecal chemotherapy, previous whole brain irradiation, serum bilirubin level, receipt of acyclovir, and receipt of ganciclovir and/or foscarnet, did not significantly alter the relationship between BC3 and encephalitis and HHV-6 and encephalitis. Three of these variables, however, were themselves associated with encephalitis after adjustment for receipt of BC3: previous intrathecal chemotherapy (risk ratio [RR], 3.7; 95% CI, 1.6–8.8), previous whole brain irradiation (RR, 2.9; 95% CI, 1.2–6.6), and serum bilirubin level (RR, 1.4 for each increase of 10 U; 95% CI, 1.2–1.6). In addition, acyclovir was associated with a decreased risk of encephalitis.

**Table 2.** Odds of reactivation of human herpesvirus 6, Epstein-Barr virus, and cytomegalovirus in BC3 recipients versus control patients, adjusted for time from bone marrow transplantation.

<table>
<thead>
<tr>
<th>Virus</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>Human herpesvirus 6</td>
<td>2.5 (1.3–4.7)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Epstein-Barr virus</td>
<td>5.1 (2.5–10.6)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Cytomegalovirus</td>
<td>1.3 (0.6–2.0)</td>
<td>.51</td>
</tr>
</tbody>
</table>

**NOTE.** Reactivation was defined as detection of >50 copies of viral DNA per mL of serum. Qualitative results did not change if the definition of reactivation was >500 or >5000 copies of viral DNA per mL of serum.
Figure 2. Human herpesvirus 6 (HHV-6) reactivation and development of encephalitis after bone marrow transplantation. A, Proportion of BC3 recipients and control patients with HHV-6 reactivation after bone marrow transplantation. B, Probability of encephalitis occurring in BC3 recipients and control patients after bone marrow transplantation.

disruption after adjustment for receipt of BC3 (RR, 0.3; 95% CI, 0.1–0.8).

DISCUSSION

Our study contributes to the accumulating evidence of the neuroinvasive potential of HHV-6 among allogeneic BMT recipients. Although the retrospective design of this study has limitations, our data suggest that the BC3 monoclonal anti-CD3 antibody and high-level HHV-6 reactivation are risk factors for encephalitis after transplantation.

Before the discovery of HHV-6, it was recognized that exanthem subitum or roseola, the clinical syndrome now known to be associated with HHV-6 primary infection, was often accompanied by neurological findings such as irritability, lethargy, febrile seizures, encephalitis, and hemiplegia [31–33]. Since the development of technology to isolate and detect HHV-6, primary infection in children has been associated with encephalitis in a number of case reports [5, 7–9, 11, 34, 35] and as a frequent cause of first-time febrile seizures [4, 36], but whether HHV-6 is associated with recurrent febrile seizures is controversial [37, 38]. HHV-6 has been associated with demyelinating disease in adult patients with AIDS [39], and a higher frequency of infection of neural cells with HHV-6 has been shown among children who have died with AIDS encephalopathy than among control patients [40]. An association between HHV-6 and multiple sclerosis has been described by means of serum antibody detection, PCR of serum and CSF, and cellular localization of virus to affected areas of the brain [41–45]. Whether this is a causal relationship remains controversial.

We used detection of viral DNA in serum as an indicator of virus reactivation. HHV-6 viremia, the ability to culture the virus from the blood or detect the viral DNA in serum, is correlated with primary HHV-6 infection and immunocompromised states [46–49]. This suggests that detection of viral DNA in hematopoietic stem cell transplant recipients reflects active viral replication and reactivation of the virus [50]. When defined by viremia, HHV-6 reactivation has been shown to occur in 36%–46% of BMT recipients [12–14] and has been associated with encephalitis in a number of case reports [15–20]. A study of 22 BMT recipients with encephalitis and 107 immunocompromised patients without CNS symptoms revealed that persons with encephalitis were more likely than control patients to have HHV-6 DNA isolated from their CSF (23% vs. 0.9%; P < 0.001) [21].

Receipt of acyclovir in our study was found to be associated with a decreased risk of encephalitis. In vitro data suggest that HHV-6 is susceptible to ganciclovir and foscarnet but only relatively susceptible to acyclovir [51, 52]. It is possible that acyclovir may exert a partial suppressive effect on HHV-6 rep-
HHV-6 reactivation was not a perfect predictor of encephalitis in our study. There were subjects with HHV-6 reactivation who did not develop encephalitis and cases of encephalitis without documentation of HHV-6 reactivation. Because of the retrospective nature of this study, we did not attempt to exclude cases of encephalitis that likely were due to known etiologies, such as bacterial, fungal, or other viral pathogens or other noninfectious etiologies. It is possible that had we used a definition of encephalitis that was more likely to have been caused by HHV-6 (e.g., encephalitis of unknown etiology), HHV-6 reactivation in serum would have been an even better predictor of encephalitis. In addition, because we relied on stored specimens to determine whether HHV-6 reactivation occurred, it is possible that we missed HHV-6 reactivation in some persons with encephalitis because we lacked samples from the period of virus reactivation. With more frequent and regular assaying for HHV-6, more cases of encephalitis might have been found to be associated with reactivation of the virus. In addition, a level of HHV-6 reactivation highly predictive of HHV-6 encephalitis may have been identified. Finally, it is possible that HHV-6 may be responsible for other clinical diseases after bone marrow transplantation, including pneumonitis [60] and bone marrow suppression [61, 62]. We did not evaluate these outcomes in this study, but HHV-6 reactivation may be an indicator of any one of a number of clinically relevant diseases in BMT recipients.

In summary, we found that both BC3 therapy and HHV-6 reactivation were independently associated with the development of encephalitis after bone marrow transplantation. The data presented here do not definitively establish a causal relationship between BC3, HHV-6 reactivation, and encephalitis after BMT, but they do provide a rationale for a prospective study of BMT recipients to better understand HHV-6 reactivation and disease. Furthermore, when allogeneic BMT recipients with neurological findings are evaluated, HHV-6 encephalitis needs to be considered in the differential diagnosis.

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