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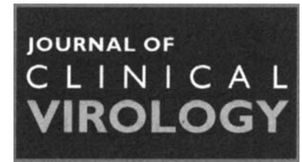

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Case report

A novel mutation of varicella-zoster virus associated to fatal hepatitis

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Abstract

Background: Lethal varicella in immunocompetent hosts is rare and its pathogenesis is largely unknown. The discovery of glycoprotein E (gE) mutants showing attributes consistent with increased virulence in vitro and in animal models, provided a possible molecular mechanism underlying a more aggressive virus infection. However, these mutants have never been associated with unusually severe clinical cases.
Objectives: To varicella-zoster virus (VZV) mutations that correlate with increased virulence.
Results: We report a case of fatal hepatitis caused by a VZV bearing a novel mutation on the 3B3 monoclonal antibody epitope of gE in an immunocompetent host.
Conclusions: This report describes a mutant VZV responsible for an aggressive clinical course in an immunocompetent host. Linking these severe clinical presentations of VZV infection to virus mutations might provide insights into the underlying pathogenic mechanisms.
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Keywords: Varicella hepatitis; VZV thymidine kinase mutant virus; Severe varicella

1. Introduction

Varicella is a common and usually benign, self-limiting disease, although serious complications are well known. Adulthood or immunologic impairment are predisposing factors for severe varicella. Varicella-zoster virus (VZV) is frequently associated with mild hepatitis (Williams and Riordan, 2000), whereas acute liver failure is rare, though often fatal. Despite such evidence, the pathogenic mechanisms underlying these complications are largely unknown. Recently, mutations of VZV DNA have been described in immunodepressed patients, although not associated with severe clinical manifestations (Santos et al., 1998; Tipples et al., 2002). We describe a case of acute, lethal liver failure in a healthy 15-year-old boy with VZV infection. Polymerase chain reaction (PCR) analysis of viral DNA extracted from autopsy tissue revealed a mutation in the ectodomain of the viral gE glycoprotein that could account for the fatal clinical presentation.

2. Case report

A 15-year-old boy without significant medical history was admitted to the infectious disease ward for evaluation of severe abdominal pain, fever, myalgia and cutaneous vesicles suggestive of varicella. The child was from a small town close to Rome, Italy. It was not possible to establish the source of varicella infection. Laboratory investigations showed the following results: Hb 15.6 g/dL; Hct 45%; WBC count 10,200 μL^{-1} (neutrophils 74%; lymphocytes 24%; monocytes 0.3%; eosinophils 0.5%; basophils 0.2%); platelets count 33,500 μL^{-1}; prothrombin time 3.12 international normalized ratio (INR); d-dimers 4975 ng/mL; alanine aminotransferase (ALT) 3715 UI/L; aspartate aminotransferase

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(AST) 3478 UI/L; lactate dehydrogenase (LDH) 4790 UI/L. Markers for hepatitis viruses were negative. Anamnestic recall excluded any drug intake, including aspirin or other anti-inflammatory drugs. X-ray of thorax was normal.

Treatment with intravenous acyclovir (ACV) 10 mg/kg three times/day was started at once along with cyprofloxacine 400 mg/day i.v. A cycle of molecular adsorbent re-circulating system (MARS) was performed. On day 3 the patient was transferred to our Intensive Care Unit (ICU) for respiratory distress. Arterial blood gas values obtained at that time were compatible with mild hypoxemia and metabolic acidosis with respiratory compensation. A new chest X-ray revealed a localized accentuation of broncho-vascular interstitium. Non-invasive ventilation was performed with helmet with beneficial effects. On admission to the ICU his neurologic status was normal, while liver function tests worsened: AST 8170 UI/L; ALT 6610 UI/L; lactate 110 mg/dL; LDH 7720 UI/L; ammonia 122 μg/dL (maximum level: 75 μg/dL); factor V activity 4% (normal 60–125%); platelets 33,000 μL^{-1}; INR 6.08; d-dimer 78,050 ng/mL; WBC count 18,095 μL^{-1}. HIV infection was excluded by RT-PCR. A hematologist ruled out blood malignancy. Daily cycles of MARS were performed. On day 6 his condition worsened, hemorrhagic vesicles spread all over the body, and signs of encephalopathy appeared. Pulmonary gas exchange deteriorated and hemodynamic status became unstable, leading to intubation and vasopressive support. Packed cells, fresh frozen plasma and platelets were transfused. On day 8 the patient died despite massive support and cardiopulmonary resuscitation.

Histopathologic examination of the liver showed panacinar necrosis and large areas of hemorrhage. In rare zones of preserved liver, eosinophilic Cowdry A—like intranuclear inclusions and multinucleation were found. The absence of microvesicular steatosis and ballooning degeneration of hepatocytes ruled out Reye's syndrome. Necrosis was present in pancreas and spleen. Furthermore, the spleen showed reactive follicular hyperplasia associated with congestion, diffuse immunoblastic and plasmacytic proliferation and outpouring of neutrophils in the red pulp. Pneumonia and myocarditis were present.

DNA was extracted (QIAgen DNA mini kit QIAGEN, GmbH, Germany) from paraffin embedded sections of liver, lungs, cardiac muscle and pancreas. Nested PCR targeting the DNA of herpes viruses (CMV, VZV, EBV, HHV6, HHV8, HSV1 and HSV2) and HBV along with RT-PCR for HCV was performed. The PCR gave negative results for all but VZV DNA, which was present in all organs tested. Sequence analysis of the VZV glycoprotein E (gE) and thymidine kinase (TK) genes was performed. Analysis of the gE revealed an amino acid change at position 161 (D–G) of the epitope 3B3 (Fig. 1A). TK analysis showed a polymorphism at position 288 of ORF 36 that changes serine to leucine. This polymorphism has been observed in several VZV strains and does not alter TK function (Morfin et al., 1999). Sequencing of the TK revealed seven novel mutations at the ATP-binding site (Fig. 1B). BLAST analysis (http://www.ncbi.nlm.nih.gov/BLAST) showed a high degree of homology with the strain isolated in Vancouver, British Columbia, Canada—BC strain (Tipples et al., 2002).

3. Discussion

Atypical clinical presentations and uncommon complications of VZV infections have been described in immunocom-

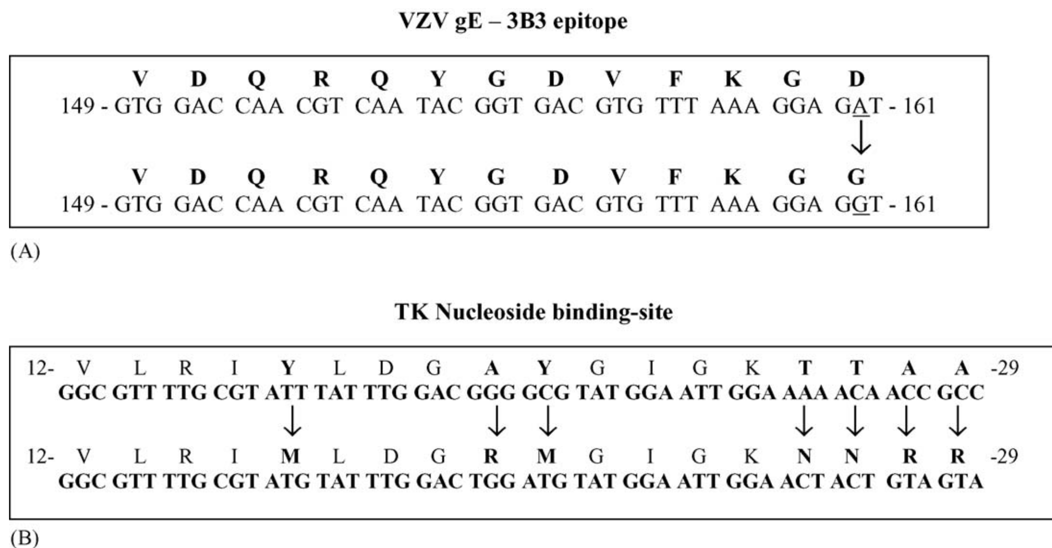

Fig. 1. (A) DNA and deduced amino acid sequence of the 3B3 epitope in the ectodomain of viral glycoprotein E (gE) in the wild type VZV (upper row) and the mutation found in our case. Sequence analysis of the gE revealed an amino acid change at position 161 (D–G) of the epitope 3B3 (underlined and arrows) (D: aspartic acid; G: glycine). (B) DNA and deduced amino acid sequence of VZV thymidine kinase at the ATP-binding site (wild type on the upper row). Arrows indicate amino acid changes from position 12 to 29 of the protein. Viral DNA was extracted from autopsy sections of the liver.

promised patients. Impaired immunity represents the main predisposing factor to the development of lethal VZV infections (Patti et al., 1990; Soriano et al., 1992; Dits et al., 1998; Gnann, 2002). Moreover, higher morbidity from varicella is described during pregnancy and adulthood, although the reasons for this are unclear. Recently, two mutant viruses able to elude immune detection were described (Santos et al., 1998; Tipples et al., 2002). These two strains bear a mutation (D150N) in the predominant glycoprotein specified in infected cells and virions (gE) that leads to the loss of a B-cell epitope. These mutant viruses grow more rapidly in cell culture than the wild type (Grose et al., 2004). Likewise, in the SCID-hu mouse (severe combined immunodeficient mouse with human skin implant) infection proceeds with increased virulence (Santos et al., 2000). These two mutant viruses were closely related genetically and were both isolated in North America. Single nucleotide polymorphism studies showed segregation of viral isolates of Asian origin from those of Western ancestry into distinct phylogenetic clades (Wagenaar et al., 2003). The VZV strain reported here, based on sequence analysis, is closely related to the Northern American BC strain. We found a novel mutation on the B-cell epitope in the gE ectodomain (Fig. 1A) described in the previously reported mutants. This mutation was in the last codon of the epitope and changes an aspartic acid to a glycine, namely a charged amino acid to a neutral one. As the epitope is a 13 amino acid sequence, it is reasonable that such a relevant mutation could be of dramatic impact on the binding affinity between epitope and antibody. It is likely that this D161G mutation alters virus–host interaction not only by avoiding the immune response, but also by changing the nature of infection at the cellular level, as described in vitro in the D150N mutants.

Two considerations emerge from these data. First, we describe a severe and atypical varicella in an immunocompetent host. Second, as already suggested (Santos et al., 1998) it appears of great interest to address the role of antibodies in VZV primary infection.

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