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DA virus mutant H101 has altered CNS pathogenesis and causes immunosuppression

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ABSTRACT

Viruses use various mechanisms to evade clearance by the host. Investigating how a few changes in the genome of a non-lethal virus can lead to altered disease, from survivable to immunosuppression/death, would provide valuable information into viral pathogenesis. The Daniels strain of Theiler's murine encephalomyelitis virus causes an asymptomatic infection or acute encephalitis followed by viral clearance. A mutant, H101, carries several alterations in the viral genome. H101 infection causes profound immunosuppression and death. Thus, a virus that is normally cleared by its natural host can become lethal due to just a few changes in the viral genome.

1. Introduction

Infections with picornaviruses are often asymptomatic. Mild illnesses (gastrointestinal infection, colds) can be caused by some picornaviruses and, on occasion, more severe diseases, such as encephalitis (inflammation in the brain), can result. *Picornaviridae* family members include the polioviruses, Saffold virus (SAFV), Foot-and-mouth disease virus (FMDV) and the hepatitis A virus (Jones et al., 2007; Nielsen et al., 2012; Tapparel et al., 2013). Recently, the picornaviruses: cosavirus, salivirus and SAFV have been detected in patients presenting with a wide range of symptoms, including gastroenteritis (Chiu et al., 2008; Blinkova et al., 2009; Chiu et al., 2010).

The newly emerged SAFV currently has 11 genotypes worldwide, SAFV-1 to -11, all of which were recently isolated from fecal specimens collected in 2009 from 943 patients with acute flaccid paralysis in Pakistan and Afghanistan (Naeem et al., 2014). Multiple SAFV strains represent all but one of the genotypes, SAFV-10, which is currently represented by only one strain. The genome diversity of SAFV was examined by comparing the full genome sequence of a representative strain from each SAFV genotype. High genetic diversity and extensive recombination were demonstrated among different SAFV genotypes (Naeem et al., 2014). SAFV is the first virus of the *Cardiovirus* genus found to infect humans, and it is closely related to the mouse pathogen Theiler's murine encephalomyelitis virus (TMEV) (Chiu et al., 2008).

TMEV belongs to the *Picornaviridae* family and *Cardiovirus* genus. The genome of TMEV is a non-enveloped, single-stranded, positive-sense RNA of approximately 8100 nucleotides which is enclosed in a highly structured capsid. The RNA genome consists of a viral protein (VPg) covalently linked to the 5′ untranslated region (UTR), an open reading frame that encodes a polyprotein, from which viral proteases post-translationally cleave 10–12 proteins, including 4 capsid proteins, followed by the 3′ UTR and a poly A tail (Rueckert, 1996). TMEV infection of mice through the natural route of infection (enteric) is mostly benign. However, rarely TMEV is able to enter into the central nervous system (CNS) and infection can lead to acute encephalitis. Mice of specific genetic backgrounds demonstrate varying susceptibilities to disease induced by TMEV (Lipton and Dal Canto, 1979). More specifically, C57BL/6 (B6) mice infected intraperitoneally (i.p.) with the Daniels (DA) strain of TMEV do not show any overt signs of disease, but an abundant virus-specific $CD8^+$ T cell response develops early following infection (Dethlefs et al., 1997a, 1997b). B6 mice infected with the DA strain of TMEV via the intracerebral (i.c.) route develop acute encephalitis; mice survive the acute disease and clear the virus by approximately day 21 post infection (p.i.), and the mice are immune to subsequent DA virus infections (Fiette et al., 1993). In comparison, SJL/J mice i.c. infected with the DA strain of TMEV are unable to clear the virus and subsequently present with chronic demyelinating disease, similar to multiple sclerosis (MS).

A mutant of the DA strain of TMEV, called H101, was inadvertently created as a result of transcription error(s) by the T7 polymerase while using a modified full-length infectious cDNA clone of the DA strain of TMEV as template (Zurbriggen et al., 1991). The H101 mutant virus encodes a point mutation (Thr101Ile) in viral protein (VP) 1, which is a capsid protein. In addition, in sequencing the H101 viral

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genome, there were also several nucleotide substitutions in the 5′ UTR as well as additional amino acid substitutions in the capsid protein coding region, suggesting that there are a number of perturbations in the viral genome (Tsunoda et al., 1997). B6 mice infected with the H101 mutant virus experienced profound immunosuppression (i.p. and i.c. routes of infection). Following i.c. infection of B6 mice, H101 viral antigen was not detected in the CNS, but infection led to greater than 90% mortality by day 7 p.i., suggesting that the H101 mutant virus led to a markedly different pathogenesis when compared to the parental DA strain of TMEV.

In this study, we provide a model of a murine virus (DA strain of TMEV) which, in the B6 mouse, can result in an asymptomatic infection and viral clearance (i.p. infection) or acute encephalitis and viral clearance (i.c. infection), but with just a few changes to the viral genome (H101 mutant virus) can result in immunosuppression (i.p. infection) and immunosuppression and death (i.c. infection). There are very few nucleotide differences between the DA strain of TMEV and the H101 mutant virus, therefore, this study provides experimental evidence that a virus that is normally cleared by its natural host can become lethal due to just a few changes to the viral genome.

2. Materials and methods

2.1. Animals

Male B6 mice and female SJL/J mice at 4- to 5-weeks of age were obtained from the Jackson Laboratory (Bar Harbor, ME). All animal experiments were conducted in accordance with the guidelines prepared by the Committee on Care and Use of Laboratory Animals, Institute of Laboratory Animals Resources, National Research Council.

2.2. Viral infection

B6 mice were anesthetized with isofluorane by inhalation and infected i.c. or i.p. with the indicated plaque forming units (pfu) of the DA strain of TMEV, the H101 (H for hydrocephalus) strain of TMEV (Tsunoda et al., 1997) or mock infected with phosphate-buffered saline (PBS) at a final volume of 20 μl per mouse. The DA and H101 strains of TMEV were propagated as previously described (Tsunoda et al., 1997).

Anesthetized B6 mice were i.c. infected with 50 pfu of lymphocytic choriomeningitis virus (LCMV), Armstrong (Arm) strain (obtained from Dr. Buchmeier, UC Irvine). Viral stocks were prepared by passaging the virus in BHK-21 cells. Viral titer was determined by plaque assay using Vero cells.

2.3. Mortality

B6 mice were infected with increasing viral doses (3×10^3, 3×10^4, 3×10^5 pfu) of H101 by i.c. inoculation. Mice were weighed and assessed for mortality through day 7 p.i.

B6 mice were infected with 3×10^5 pfu of H101 by i.p. inoculation, followed by 50 pfu of LCMV (Arm) by i.c. inoculation on day 7 post-H101-infection. Mice were assessed for mortality through day 7 post-LCMV-infection (day 14 post-H101-infection).

2.4. Spleen weights

B6 mice were infected with 3×10^5 pfu of DA or H101 virus, or mock infect, by i.c. inoculation. Spleens were harvested from infected mice on days 3 and 5 p.i. and weighed.

2.5. Immunohistochemistry

B6 mice, i.c. infected with 3×10^5 pfu of DA or H101 virus, or mock infect, were euthanized on day 3 p.i. Animals were perfused with PBS followed by 4% paraformaldehyde. Brains were harvested, divided into five coronal slabs and embedded in paraffin. Four-μm-thick tissue sections were cut and mounted onto slides. Viral antigen-positive cells were detected on paraffin sections using TMEV hyperimmune rabbit serum, as previous described (Zurbriggen and Fujinami, 1989; Tsunoda et al., 2001). Reactivity of the TMEV hyperimmune rabbit serum for H101 viral antigens was demonstrated previously (Tsunoda et al., 1997). Viral antigen-positive cells were enumerated and summed, in a blinded fashion, using one slide per brain and evaluating the hippocampal region of the tissue sections.

B6 mice, i.p. infected with 3×10^5 pfu of H101 followed by i.c. infection with 50 pfu of LCMV (Arm) on day 7 post-H101-infection, or mono-infected with either H101 or LCMV, were euthanized on day 7 p.i. Animals and tissues were processed as described above. CD8 antigen-positive cells were detected on paraffin sections using anti-mouse CD8-β antibody (Santa Cruz Biotechnology, Santa Cruz, CA). The tissues were visualized using the avidin–biotin peroxidase complex technique with 3,3′-diaminobenzidine tetrahydrochloride (Sigma, St. Louis, MO) in 0.01% hydrogen peroxide (Sigma) in PBS. The tissues were then counterstained with Harris' hematoxylin (Electron Microscopy Sciences, Hatfield, PA).

SJL/J mice sensitized with myelin proteolipid protein (PLP) 139–151 peptide (see Section 2.9. below) which were subsequently i.p. infected with 3×10^5 pfu of H101 on day 19 post sensitization were euthanized following the first relapse. Animals were perfused with PBS followed by 4% paraformaldehyde. Spinal cords were harvested, divided into 12 transverse portions and embedded in paraffin. Four-μm-thick tissue sections were cut and mounted onto slides. CD3 antigen-positive cells were detected on paraffin sections using anti-mouse CD3ε antibody (BD Bioscience, San Jose, CA) following antigen retrieval. The tissues were visualized and counterstained as described above. Each spinal cord cross section was visually divided into quarters: top, bottom, right and left. CD3 antigen-positive cells were enumerated within each available quarter, all available quarters were summed and the sum was divided by the total number of available quarters to obtain a pathologic score for each mouse.

2.6. Flow cytometry

Flow cytometry was performed as previously described (Cusick et al., 2013). Briefly, B6 mice, i.c. infected with 3×10^5 pfu of DA or H101 virus, or mock infected, were euthanized and perfused with PBS on day 3 p.i. Subsequently, cells were mechanically isolated from the spleens and suspended in RPMI 1640 medium (Mediatech, Herndon, VA) supplemented with 1% L-glutamine (Mediatech), 1% antibiotics (Mediatech), 50 μM 2-mercaptoethanol (Sigma), and 10% Cosmic Calf Serum (CCS; Hyclone, Logan, UT). Cells were further purified with Histopaque-1083 (Sigma). Cells were treated with Fc block (BD Bioscience), stained with the indicated anti-mouse antibodies for 30 minutes at 4 °C [anti-CD45–v500 and anti-CD3ε–allophycocyanin (APC)-Cy7, (obtained from BD Bioscience); anti-CD19–v450 and anti-CD11b–APC (obtained from eBioscience, San Diego,CA)], and analyzed by flow cytometry on a BD FACSCanto II (BD Biosciences). Brain-derived cells were stained and analyzed individually for each mouse. Gating was determined based on fluorescence-minus-one (FMO) with isotype matched immunoglobulin (Ig) controls. More specifically, FMO controls contained each antibody conjugate used in the experiment except one, with the addition of the appropriate isotype control for the excluded fluorochrome. This was performed for each fluorochrome. Flow cytometry data analysis was performed using FlowJo software (Tree Star, Inc., Ashland, OR).

2.7. Mitogen-induced proliferation assays

Spleens were harvested, on day 7 p.i., from B6 mice i.p. infected with increasing viral doses of H101 (1×10^1, 1×10^2, 1×10^3, 1×10^4, 1×10^5 pfu). Mononuclear cells were isolated with Histopaque-1083.