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Rescue of wild-type mumps virus from a strain associated with recent outbreaks helps to define the role of the SH ORF in the pathogenesis of mumps virus

Pei Xu [a,b], Zhuo Li [a], Dengyun Sun [a,b], Yuan Lin [a], Jianguo Wu [c,d], Paul A. Rota [e], Biao He [a,c,*]

[a] Department of Infectious Diseases, College of Veterinary Medicine, University of Georgia, GA 30602, USA
[b] Intercollege Graduate Program in Cell and Developmental Biology, Pennsylvania State University, University Park, PA 16802, USA
[c] State Key Laboratory of Virology, College of Life Sciences, Wuhan University, Wuhan 430072, P.R. China
[d] Chinese-French Liver Disease Research Institute at Zhongnan Hospital, Wuhan University, Wuhan 430072, P.R. China
[e] Centers for Diseases Control and Prevention, Atlanta, GA 30333, USA

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ABSTRACT

Mumps virus (MuV) causes acute infections in humans. In recent years, MuV has caused epidemics among highly vaccinated populations. The largest outbreak in the U.S. in the past 20 years occurred in 2005–2006 with over 5000 reported cases in which the majority of the cases was in vaccinated young adults. We sequenced the complete genome of a representative strain from the epidemic (MuV-IA). MuV-IA is a member of genotype G, the same genotype of MuV that was associated with the outbreak in the UK in 2004–2005. We constructed a reverse genetics system for MuV-IA (rMuV-IA), and rescued a virus lacking the open reading frame (ORF) of the SH gene (rMuVΔSH). rMuVΔSH infection in L929 cells induced increased NF-κB activation, TNF-α production and apoptosis compared to rMuV-IA. rMuVΔSH was attenuated in an animal model. These results indicated that the SH ORF of MuV plays a significant role in interfering with TNF-α signaling and viral pathogenesis during virus infection.

Introduction

Mumps virus (MuV) is a member of the family *Paramyxoviridae*. MuV causes acute parotitis in humans, characterized by lateral or bilateral swelling of the salivary glands (Carbone and Wolinsky, 2001). MuV is also notable as a neurotropic agent causing a number of central nervous system (CNS) manifestations ranging from mild meningitis to severe encephalitis. The incidence of mumps and its complications were dramatically reduced following the introduction of measles, mumps, rubella vaccine (MMR) in 1971 (Amexis et al., 2002; Carbone and Wolinsky, 2001). MMR vaccine containing the Jeryl Lynn (JL) strain, an attenuated strain of MuV, is highly efficacious and produces few adverse reactions.

In spite of high coverage with MMR, mumps outbreaks continue to occur in the US and other countries. The causes cited range from failure to vaccinate, vaccine failure, and emergence of strains of MuV capable of escaping vaccine-induced immunity (Crowley and Afzal, 2002; Lim et al., 2003; Otto et al., 2010; Strohle et al., 1996; Utz et al., 2004; Whelan et al., 2010). In 2006, the U.S. experienced the largest mumps epidemic in nearly 20 years (Marin et al., 2008). The outbreak originated at a university in Iowa and spread to 11 other states. Over 5000 mumps cases were reported in 2006 compared to an average of approximately 250 cases/year in the previous decade. In 2009–2010, a mumps outbreak occurred in the State of New York and the State of New Jersey in the US in which 88% of the patients had one-dose of mumps vaccine and 75% of the patients had two doses of vaccine (MMWR, 2010).

The SH gene of MuV is the most variable region in mumps genome. Based on the sequence variability of the SH protein, all current wild type MuVs have been classified into 12 genotypes (Jin et al., 2005). Sequence comparison of the SH proteins from the outbreaks indicated that both Iowa and New Jersey/New York outbreaks were caused by closely related mumps strains in genotype G. Interestingly, the genotype G viruses detected in the outbreaks in the U.S. were also closely related to mumps virus strains associated with the massive mumps epidemic in the UK in which over 56,000 cases were reported in the 2004–2005 (MMWR, 2006; Rota et al., 2009; Watson-Creed et al., 2006).

MuV, a paramyxovirus, is a negative stranded, non-segmented RNA virus with a genome of 15,384 nucleotides (Carbone and Wolinsky, 2001). The virus has seven genes but encodes nine

* Corresponding author at: Department of Infectious Diseases, College of Veterinary Medicine, University of Georgia, 501 D.W. Brooks Dr., Athens, GA 30602, USA.
E-mail address: bhe@uga.edu (B. He).

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known viral proteins. The nucleocapsid protein (NP), phosphoprotein (P) and large RNA polymerase (L) protein are important for transcription and replication of the viral RNA genome (Elango et al., 1988; Okazaki et al., 1992; Rima et al., 1980). The V/P gene encodes three proteins, I, V and P (Paterson and Lamb, 1990). Mutations in the P gene have been associated with increased virulence of mumps virus (Saito et al., 1996). The V protein plays important roles in inhibiting interferon signaling in infected cells (Kubota et al., 2002; Takeuchi et al., 1990; Ulane et al., 2003; Yokosawa et al., 2002). The fusion (F) protein, a glycoprotein, mediates both cell-to-cell and virus-to-cell fusion in a pH-independent manner that is essential for virus entry into cells (Waxham et al., 1987). The hemagglutinin-neuraminidase (HN), another viral glycoprotein, is also involved in virus entry (Tanabayashi et al., 1992) and mutations in the HN gene have been implicated in mumps virus virulence (Cusi et al., 1998). The matrix (M) protein plays an important role in virus assembly (Matsumoto, 1982). The small hydrophobic (SH) protein is a 57-residue type 1, hydrophobic integral membrane protein (Elango et al., 1988). It has been reported that MuV SH has a similar function to the SH protein of a closely related virus, parainfluenza virus 5 (PIV5, formerly known as simian virus 5, SV5) (Hiebert et al., 1988; Wilson et al., 2006). While the SH ORF of PIV5 is not essential for virus growth in tissue culture cells, a virus lacking SH induces higher levels of cell death and is attenuated in an animal model (He et al., 1998, 2001). SH of PIV5 inhibits TNF-α signaling (Lin et al., 2003; Wilson et al., 2006). However, the expression of the SH protein in cells infected with MuV has never been demonstrated and the role of SH has never been reported. In this work, we have generated a reverse genetics system based on a representative isolate from the outbreak in 2006 in the U.S. and studied the biologic properties of a rescued recombinant virus lacking the SH ORF. These results indicated that MuV SH ORF plays a role in interfering with TNF-α signaling during virus infection.

Results

Sequence of the complete genome of MuV-IA

To better understand the genetic characteristics of viruses associated with recent outbreaks in the U.S., the complete genomic sequence of a representative isolate from the Iowa outbreak was determined (JN012242). A set of primers was designed based on the consensus sequence derived from comparison of the genomic sequences of Jeryl Lynn, Urabe, 88–1961 and PetroNov. Viral RNA of MuV-IA was reverse-transcribed into cDNA using random hexamers, PCR reactions were then carried out using the set of primers and the products were sequenced using the corresponding primers. A second set of primers based on the sequencing results were then used to perform RT-PCR and the products overlapping with those of first round of sequencing fragments were sequenced using the primers. Leader and trailer sequences were determined by performing 5′/3′ RACE.

There is only one conserved change in the putative transmembrane domain of the SH protein when the SH protein sequence of MuV-IA was compared to other strains of mumps virus in genotype G (Fig. 1A), confirming that MuV-IA belongs to genotype G (Rota et al., 2009). To further study the genomic divergence of MuV-IA, a phylogenetic tree was generated using the genomic sequence of MuV-IA and 32 full length genomic sequences from Genbank (Fig. 1B). Phylogenetic analysis indicated that MuV-IA is most closely related to the sequence of MuV Du/CRO05, a genotype G virus, which was isolated in Croatia in 2005 (Santak et al., 2006). A comparison of the predicted amino acid sequences between the protein coding regions of MuV-IA and Jeryl Lynn vaccine (major component) showed that while NP, M and L protein sequences are highly conserved with an identity of over 98%, there was more divergence among V, P, F, SH and HN proteins (Fig. 1C). The predicted SH protein sequences had only 85% identity.

Generation of an infectious cDNA clone for MuV-IA

To be able to study the pathogenesis of MuV-IA, a reverse genetics system was derived. Because RNA viruses exist as a quasi-species, the consensus sequence of the genome was used as the base for the recombinant MuV. A plasmid containing a mini-genome with luciferase (Luc) reporter gene for mumps virus (pT7-MuV-Mini-Luc) similar to the PIV5 mini-genome expressing plasmid was constructed using rMuV-IA trailer and leader sequences (data not shown) (Lin et al., 2005). In addition, plasmids encoding NP, P and L in the pCAGGS vector have been obtained and confirmed by sequencing. To test the functionality of the plasmids, the plasmids were transfected into BSR-T7 cells. At 2 dpi, the cells were harvested and Luciferase (Luc) assays were performed. Luc activity was detected in the cell transfected with all plasmids, not ones missing P or L, indicating that the plasmids expressed functional P and L proteins (data not shown). RT-PCR was conducted to amplify DNA fragments representing the complete genome and inserted into individual plasmid vectors before being assembled into a full-length genome. The plasmid with the full length genome of MuV-IA expressed under the control of a T7 (pMuV-IA) promoter (pMuV-IA) was similar to the plasmid used to generate infectious PIV5 (He et al., 1997). pMuV-IA had changes in two nucleotides within the L ORF compared with consensus sequence of MuV-IA at positions of 11863 (T to C) and 12028 (C to T). However, neither of these nucleotide changes resulted in changes in the predicted L protein sequence. A recombinant MuV (rMuV-IA) was rescued using the plasmid containing the full-length genome of MuV-IA. BSRT-7 cells were co-transfected with pMuV-IA and plasmids expressing viral RNA polymerase components. Individual plaques were selected and amplified in Vero cells. The entire genome of the rescued virus was sequenced and found to match the input cDNA genome sequence (data not shown).

To compare time course of the growth of rMuV and MuV-IA, a multi-cycle growth assay was performed (Fig. 2A). Both viruses grew to similar peak titers in Vero cells. Viral titers in the supernatant of the infected cells increased exponentially during the first 2 days after infection, and reached a titer of 10^7 pfu/ml at 48 hpi. We also compared the growth of both viruses in HeLa cells (a human cell line), MDBK cells (a bovine cell line), and L929 cells (a murine cell line) and did not observe any obvious differences between these two viruses (data not shown). The viral protein expression levels in cells were also examined using Western blot (Fig. 2B) and the protein levels were similar at different time points after infection, indicating that the replication of rMuV resembles MuV-IA in tissue culture cells.

In addition, we rescued infectious recombinant viruses expressing either EGFP or Renila Luciferase (RL) protein as an extra gene. pMuV-EGFP was constructed by inserting an EGFP gene, flanked by gene start (GS) of SH and gene end (GE) of NP, between F gene and SH gene in pMuV-IA, pMuV-RL was constructed through substitution of coding sequence of EGEP with that of renilla luciferase (RL) in pMuV-EGFP. Expression of EGFP or RL in the infected Vero cells was detected (Figs. 2C, D).

Rescue of a recombinant mumps virus lacking the SH ORF

To study the function of the SH protein of MuV, 156 nucleotides in the SH gene open reading frame (ORF) of the SH gene were deleted from pMuV-IA. The truncated SH ORF contained a short ORF encoding 5 amino acid residues flanked by the original SH ORF start and gene end (pMuV-IAΔSH, Fig. 3A). An infectious MuV lacking the SH ORF was rescued (rMuVΔSH) (Figs. 3B and C) and the genome was sequenced, which matched the input cDNA sequence (data not shown). The rMuVΔSH genome was of 15,228 nt in size, complying with "the rule of