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The genome sequence of ectromelia virus Naval and Cornell isolates from outbreaks in North America

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ABSTRACT

Ectromelia virus (ECTV) is the causative agent of mousepox, a disease of laboratory mouse colonies and an excellent model for human smallpox. We report the genome sequence of two isolates from outbreaks in laboratory mouse colonies in the USA in 1995 and 1999: ECTV-Naval and ECTV-Cornell, respectively. The genome of ECTV-Naval and ECTV-Cornell was sequenced by the 454-Roche technology. The ECTV-Naval genome was also sequenced by the Sanger and Illumina technologies in order to evaluate these technologies for poxvirus genome sequencing. Genomic comparisons revealed that ECTV-Naval and ECTV-Cornell correspond to the same virus isolated from independent outbreaks. Both ECTV-Naval and ECTV-Cornell are extremely virulent in susceptible BALB/c mice, similar to ECTV-Moscow. This is consistent with the ECTV-Naval genome sharing 98.2% DNA sequence identity with that of ECTV-Moscow, and indicates that the genetic differences with ECTV-Moscow do not affect the virulence of ECTV-Naval in the mousepox model of footpad infection.

Introduction

Mousepox is an acute exanthematous disease of mice caused by the orthopoxvirus (OPV) ectromelia virus (ECTV) that in the past century affected laboratory mouse colonies worldwide (Esteban and Buller, 2005; Fenner, 1981; Marchal, 1930). ECTV, like variola virus (VARV), the causative agent of smallpox, has a narrow host range and causes a severe disease with skin lesions in the later stages of the infection and a high mortality rate (Esteban and Buller, 2005). Mousepox was first described in 1930 in Hampstead (United Kingdom) as an infectious disease of mouse associated with high mortality and amputations in mice recovered from infection (Marchal, 1930). Following the identification of ECTV-Hampstead, ECTV has been isolated in temporally and geographically different laboratory mousepox outbreaks. ECTV-Moscow is a highly virulent strain isolated in Moscow in 1947 and extensively used in pathogenesis studies, and ECTV-Ishibashi, isolated in Japan in 1966, was used in the initial ECTV studies (Allen et al., 1981; Andrewes and Elford, 1947; Ichihashi and Matsumoto, 1966). Several important mousepox outbreaks occurred in the USA between the seventies and the eighties. As a consequence, tens of thousands of laboratory mice were sacrificed and millions of dollars were lost in biomedical research (Lipman et al., 1999). ECTV-NIH79 was a virulent strain isolated in 1979 at the National Institutes of Health (NIH) in Bethesda, Maryland (USA) (Allen et al., 1981). In the same period, various outbreaks were reported in biomedical research institutions in Saint Louis, Chicago, Minneapolis and Salt Lake City (Dixon, 1981; La Regina and Doyle, 1981; Wallace, 1981). Because the clinical signs of mousepox are not evident in resistant mouse strains, ECTV infections could have been present in colonies for long periods before their identification in animal house facilities in USA. The destruction of entire mouse colonies was not always satisfactory to control mousepox spread (Wallace, 1981). In order to fight this continuous threat, working with live ECTV was prohibited in the USA (Fenner et al., 1989). In 1995, the virulent strain ECTV-Naval caused an

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outbreak in the laboratory mouse colony of the US Naval Medical Research Institute in Bethesda, Maryland (USA) (Dick et al., 1996). The outbreak caused devastating effects and ended with the sacrifice of thousands of mice, affecting more severely the BALB/c mice (Dick et al., 1996). The last outbreak of mousepox was reported in 1999 at the Weill Medical College of Cornell University in New York (USA), and was successfully contained and eradicated from the animal house facility (Lipman et al., 1999, 2000). Contrary to initial speculations about the existence of a natural reservoir for this virus within the USA, it was found that the Cornell University outbreak originated from an injection of mice with a contaminated commercial mouse serum imported in 1995 from China, and the same origin was speculated for the outbreak in Bethesda that occurred four years earlier (Lipman et al., 1999, 2000). ECTV outbreaks have no longer been reported (Huggins et al., 2009), probably due to improved housing conditions of laboratory mouse colonies and routine testing of the health status of mouse colonies. Nearly a century after the first ECTV description, it is intriguing that this virus has never been isolated from a mouse or rodent in nature, and its natural reservoir remains unknown (Fenner et al., 1989). The only exception was a report describing a putative ECTV infection in field mice in Germany in 1962. This disease could be transmitted by footpad inoculation to laboratory white mice and produced typical symptoms of ECTV infection, including skin lesions, foot inflammation and amputation (Groppel, 1962). However, the virus was not isolated and the presence of ECTV was not confirmed by molecular techniques.

The growing fear of the possible use of VARV as a bioterrorist weapon causing an epidemic in the human population that is no longer vaccinated against the disease prompted the need for the development of safer smallpox vaccines (Smith and McFadden, 2002). Moreover, OPV zoonoses have increased in recent years; monkeypox virus (MPXV) may occupy the niche vacated by VARV in Africa and cowpox virus (CPXV) is transmitted to humans by domestic animals and occasionally wild rodents in Europe (Favier et al., 2011; Giulio and Eckburg, 2004; Wolfs et al., 2002). The major limitation for the improvement of the current repertoire of poxvirus vaccines is the lack of accessible human OPV infections for clinical efficacy trials. The genetic similarity of ECTV to VARV, CPXV and MPXV, together with the convenience of a laboratory mouse model, underscores the utility of mousepox as a model for the study of OPV infections (Buller, 2004).

The recent development of next generation sequencing technologies has had a pronounced impact on virology providing a fundamental tool for sequencing large viral genomes (Afonso et al., 2012; Lin et al., 2013; Mavian et al., 2014, 2012a, 2012b; Qin et al., 2013) for metagenomic studies of viral communities (Lopez-Bueno et al., 2009; Reyes et al., 2010) and also for the identification of new viruses and pathogens (Radford et al., 2012). In spite of the relevance of the mousepox model, only two ECTV genomes have been sequenced: ECTV-Moscow and Erythromelalgia-related poxvirus (ERPV), an ECTV strain isolated in China from throat specimen of individuals affected by erythromelalgia, a vascular disease of extremities and characterized by hyperthermia and vasodilatation (Chen et al., 2003; Mendez-Rios et al., 2012; Zheng et al., 1992, 1988). Here we report the sequence of the ECTV-Naval and ECTV-Cornell genomes determined by three different sequencing technologies (Sanger, 454-Roche and Illumina) and define the virulence of these isolates in susceptible mice.

Results

Plaque purification of ECTV-Naval.Cam

The plaque-purified ECTV-Naval.Cam stock was obtained after three rounds of plaque purification of a single plaque derived from the spleen of a BALB/c mouse infected with the original stock of the Naval Medical Research Institute outbreak (Bethesda, Maryland, USA) (Dick et al., 1996). The virulence in susceptible BALB/c mice of ECTV-Naval.Cam is similar to that of the original uncloned stock, indicating that the plaque-purified virus has not been attenuated and is representative of the parental virus isolate (data not shown). ECTV-Naval.Cam is referred in the text as ECTV-Naval.

Sequencing of the ECTV-Naval genome

The genome of ECTV-Naval has been sequenced by three different sequencing technologies: Sanger, 454-Roche and Illumina. Sequences obtained by Sanger (5810 reads with an average length of 536 bp) were assembled into five contigs accounting for 202 Kbp. Further 42 reads were obtained to close gaps and to raise the quality of low coverage regions. Finally, a contiguous linear sequence of 207,620 bp with 13 × coverage was attained (Table 1). The leftmost nt was an arbitrarily designated base one. The ITR of the genome contains the OPV specific structures: the hairpin loop, the concatemer resolution motif, two sets of direct repeats (DRI and DRII) and the non-repetitive I (NRI) and NRII sequences flanking DRI. The DRI of the ECTV-Naval genome, as for ECTV-Moscow, is formed by a unique sequence of 68 bp, in comparison with the 69 bp sequence repeated 2.3 × of ERPV (Chen et al., 2003; Mendez-Rios et al., 2012). DRII contains an 85 bp sequence repeated 19.4 ×, instead of 10.4 × found in the ECTV-Moscow and ERPV genomes (data not shown) (Chen et al., 2003; Mendez-Rios et al., 2012). A third set of direct repeats, located in the central region of the genome (DRIII), contained a 24 bp ("TCTATATCCTG-TACTATACCATTA") sequence repeated 25 ×. Although the hairpin loop structure was not experimentally isolated and sequenced, the comparison by clustalw2 alignment with the sequence of the termini of the ECTV-Moscow and ERPV genomes suggested that the genome was represented completely in the sequence, including the hairpin loop structures.

The 454-Roche sequencing output consisted of 92,155 reads with an average length of 360 bp (Table 1). The 454-Roche reads were assembled into two contigs of approximately 35 Kbp and 163 Kbp, separated by the DRIII region. Sanger sequencing of a PCR product including the DRIII region also failed to determine the exact number of repeats. We estimated by gel electrophoresis of the PCR product the presence of 17 repeats of 24 bp in the DRIII region (Fig. 1). The genome sequence obtained by 454-Roche has a size of 204,205 bp, including a DRII region of 85 bp sequence repeated only 2.3 × (Table 1). The coverage of the genome was 83 ×, almost seven times higher than the coverage obtained by Sanger. This 454-Roche genome showed eight differences when compare to the genome obtained by Sanger, mostly due to A/T homopolymeric regions.

Table 1
DNA sequencing of the ECTV-Naval and the ECTV-Cornell genomes.

	ECTV-Naval			ECTV-Cornell
	Sanger	454-Roche	Illumina	454-Roche
Total reads	5810	92,155	5,497,834	67,965
Mapped reads (%)[a]	nd	45,195 (49.0%)	4,835,589 (87.9%)	58,558 (86.1%)
Average reads size (bp)	536	360	76	384
Genome coverage (x)	13	83	1770	115
Genome size (bp)	207,620	204,099	207,620	204,499
Differences with Sanger genome [b]	–	8	0	nd

nd, not determined.

[a] Number of reads mapped to the reference viral genome .

[b] Number of nucleotide differences as compared to the Sanger genome.