CONSTRUCTION OF A FULL-LENGTH cDNA INFECTIOUS CLONE OF A EUROPEAN-LIKE TYPE 1 PRRSV ISOLATED IN THE U.S.

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1. INTRODUCTION

The recent emergence of a unique group of European-like Type 1 porcine reproductive and respiratory syndrome virus (PRRSV) isolates in the U.S. presents new diagnostic and disease control problems for a swine industry that has already been seriously impacted by the traditional North American Type 2 PRRSV. Genetic and antigenic analysis from our laboratories demonstrated that this group of U.S. Type 1 PRRSV has features that distinguish it from typical European Type 1 PRRSV.[1,2] In order to further characterize this group of U.S. Type 1 PRRSV and provide an essential tool for the future construction of a new generation of genetically engineered PRRSV vaccines for both Type 1 and Type 2 PRRSV, we constructed a full-length cDNA infectious clone of a U.S. Type 1 PRRSV. This is the first Type 1 infectious clone shown to replicate well in MARC-145 cells and represents the second infectious clone of Type 1 PRRSV. In addition, this infectious clone represents a recent member of this genotype, differentiating itself from the Lelystad infectious clone[3] derived from a 15-year-old strain of PRRSV.

2. MATERIALS AND METHODS

A European-like Type 1 PRRSV isolate, SD 01-08 (P34) was used for construction of a full-length infectious clone. SD 01-08 was isolated in 2001 from a group of 8-week-old pigs showing no clinical signs. BHK-21 cells were used for initial transfection, and MARC-145 cells were used for virus rescue and subsequent experiments.

To construct the full-length cDNA clone, seven overlapping fragments (except the 5' and 3' ends) flanked by unique restriction enzyme sites were amplified by RT-PCR and

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cloned into the pCR-Blunt II-Topo vector. These fragments were assembled into the low copy number plasmid, pACYC177, by restriction enzyme digestion, ligation, and transformation. The 5' and 3' ends of the genome were determined using a GeneRacer kit (Ambion) and assembled into pACYC177 vector. To rescue infectious virus, capped RNA was transcribed *in vitro* from the pACYC177 clone and transfected into BHK-21C cells using DMRIE-C (Invitrogen). Cell culture supernatant obtained 48 hours post-transfection was serially passaged on MARC-145 cells. Rescue of infectious virus was confirmed by immunofluorescent assay (IFA) using Type 1 and Type 2 PRRSV Nsp2 and N specific monoclonal antibodies (MAbs). For discrimination between the cloned virus and parental SD 01-08 virus, a *ScaI* restriction enzyme site was engineered into the ORF7 region of the cloned virus using site-directed mutagenesis.

Growth kinetics was examined by infecting MARC-145 cells with cloned virus and parental virus at a MOI of 0.1. Infected cells were collected at various times post-infection, and the virus titers were determined by IFA on MARC-145 cells and expressed as fluorescent focus units per ml (FFU/ml). Plaque morphology between the cloned virus and parental virus was compared by plaque assay on MARC-145 cells.

3. RESULTS AND DISCUSSION

A full-length genomic cDNA clone of a European-like (U.S. Type 1) PRRSV, strain SD 01-08 was constructed. This construct contains a bacteriophage T7 RNA polymerase promoter at the 5' terminus of the viral genome, an additional guanosine residue introduced between the T7 promoter and the first nucleotide of the viral genome, 15047 nucleotides full-length genome of SD 01-08 and a poly (A) tail of 41 residues incorporated at the 3' end of the genome.

The *in vitro* transcribed capped RNA was transfected into BHK-21 cells. Forty-eight hours post-transfection, cells were examined by IFA using nucleocapsid (N) protein specific MAb SDOW17 (Fig. 1A). Results showed that about 5% of cells transfected with pSD 01-08 RNA expressed the N protein. Supernatants from the transfected cells were passaged to naïve MARC-145 cells. After 48 hours postinfection, MARC-145 cells were tested using Type 1 PRRSV specific, anti-Nsp2 MAb ES2 36-19 (Fig. 1B), and a MAb recognizing both genotypes, SDOW 17 (Fig. 1C). A Type 2 PRRSV specific, anti-N MAb MR39 (Fig. 1D) was used as a negative control. The results showed that both Nsp2 and N proteins were detected in MARC-145 cells inoculated with supernatant from transfected BHK-21 cells. Upon further passage in MARC-145 cells (passage 2 on MARC-145 cells), cytopathic effects (CPE) were observed within 48 to 72 hours post-infection. These results indicate that viable and infectious PRRSV was rescued from the cells transfected with *in vitro* transcribed RNA. The cloned virus from the second MARC-145 cell passage was also passaged on porcine alveolar macrophages (PAM). IFA results confirmed the presence of virus replication in PAM (Fig. 1E and 1F), which indicates that cloned virus possessed the ability, as its parental virus, to replicate not only in MARC-145 cells but also in PAM.

The growth properties of the cloned virus were compared with that of parental virus. Results showed that there were no significant differences in growth kinetics and plaque morphology between cloned virus and its parental virus (data not shown).

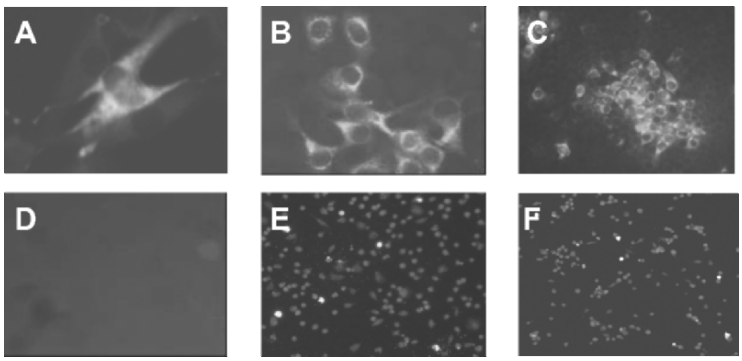

Figure 1. Rescue and passage of cloned U.S. Type 1 virus, SD 01-08. Picture A, BHK-21C cells transfected with *in vitro* transcribed RNA from the full-length cDNA clone. Pictures B, C, and D, MARC-145 cells were infected with cloned virus rescued from BHK cells. Cells were fixed and stained with PRRSV specific monoclonal antibodies (MAbs) at 48 hours post-transfection (or infection). A. Anti-N MAb SDOW17; B. Anti-Nsp2 MAb ES2 36-19 (Type 1 PRRSV specific); C. Anti-N MAb SDOW17; D. Anti-N MAb MR40 (Type 2 PRRSV specific). Pictures E and F, porcine alveolar macrophages were infected with parental virus (E) and cloned virus (F), IFA stained with anti-N MAb SDOW17.

To differentiate cloned virus from the parental virus, we engineered a *ScaI* restriction enzyme site at nucleotide 42 of ORF7. A 1057- bp RT-PCR fragment derived from the cloned virus was cleaved by *ScaI*. In contrast, the RT-PCR fragment derived from the parental isolate was not cleaved by *ScaI.*

In conclusion, we successfully constructed a full-length cDNA infectious clone of a U.S. Type 1 PRRSV. The cloned virus maintained similar *in vitro* growth properties as that of parental virus. The availability of this U.S. Type 1 infectious clone provides an important research tool to study the virulence factors and pathogenic mechanisms of PRRSV. In conjunction with the traditional North American Type 2 infectious clones,[4-7] a new generation of genetically engineered chimeric PRRSV vaccines can be constructed.

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