Veterinary Microbiology 150 (2011) 49–62

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Genetic and immunobiological diversities of porcine reproductive and respiratory syndrome genotype I strains

Laila Darwich [a,b,1,*], Mariona Gimeno [a,b,1], Marina Sibila [b], Ivan Diaz [b], Eugenia de la Torre [b], Silvia Dotti [b,2], Liudmila Kuzemtseva [b], Margarita Martin [a,b], Joan Pujols [b,c], Enric Mateu [a,b]

[a] Departament de Sanitat i Anatomia Animals, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain
[b] Centre de Recerca en Sanitat Animal (CReSA), UAB-IRTA, campus UAB, 08193 Bellaterra, Spain
[c] Institut de Recerca i Tecnologia Agroalimentària (IRTA), Barcelona, Spain

ARTICLE INFO

Article history:
Received 22 June 2010
Received in revised form 30 December 2010
Accepted 10 January 2011

Keywords:
Porcine reproductive and respiratory syndrome virus
Complete ORF sequences
Genotype-I
Phylogenetic analyses
Immunobiological properties

ABSTRACT

Genetic diversity of porcine reproductive and respiratory syndrome virus (PRRSV) has been based on ORF5/GP5 and ORF7/N protein variations. Complete viral genome studies are limited and focused on a single or a few set of strains. Moreover, there is a general tendency to extrapolate results obtained from a single isolate to the overall PRRSV population. In the present study, six genotype-I isolates of PRRSV were sequenced from ORF1a to ORF7. Phylogenetic comparisons and the variability degree of known linear B-epitopes were done considering other available full-length genotype-I sequences. Cytokine induction of all strains was also evaluated in different cellular systems. Non structural protein 2 (nsp2) was the most variable part of the virus with 2 out of 6 strains harboring a 74 aa deletion. Deletions were also found in ORF3 and ORF4. Phylogenetic analyses showed that isolates could be grouped differently depending on the ORF examined and the highest similarity with the full genome cluster was found for the nsp9. Interestingly, most of predicted linear B-epitopes in the literature, particularly in nsp2 and GP4 regions, were found deleted or varied in some of our isolates. Moreover, 4 strains, those with deletions in nsp2, induced TNF-α and 3 induced IL-10. These results underline the high genetic diversity of PRRSV mainly in nsp1, nsp2 and ORFs 3 and 4. This variability also affects most of the known linear B-epitopes of the virus. Accordingly, different PRRSV strains might have substantially different immunobiological properties. These data can contribute to the understanding of PRRSV complexity.

1. Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) emerged in the last years of the decade of 1980s and by early 1990s became one of the major pathogens affecting the swine industry. At present, some 20 years after its emergence (Keffaber, 1989; Paton et al., 1991), PRRSV and the syndrome that it causes are still little understood and fully efficacious vaccines are lacking. The reasons for such a lack of vaccines are diverse but the high genetic diversity of the virus and the ability of PRRSV for subverting the immune response of the host are paramount. PRRSV is a positive-sense ssRNA enveloped virus classified within the genus *Arterivirus*. Nowadays, two genotypes are recognized (I and II) that originally were described as European and American because of the geographic origin of their prototypic strains (Lelystad virus and VR-2332), respectively (Collins et al., 1992; Wensvoort et al., 1991). Genetic diversity within geno-

* Corresponding author at: Departament de Sanitat i Anatomia Animals, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain. Tel.: +34 935811046; fax: +34 935813297.
E-mail address: laila.darwich@uab.cat (L. Darwich).
[1] LD and MG have contributed equally to this study.
[2] Current address: Instituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "Bruno Ubertini", Via Bianchi, 9, 25124 Brescia, Italy.

0378-1135/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetmic.2011.01.008

Table 1
Characteristics of PRRSV strains used in the present study.

Strain reference	Country	Year of isolation	Clinical signs (field)	Tissue	Maximum titer in PAM* ($TCID_{50}$/ml)
2982	Spain	2005	Respiratory (weaners)	Lung (weaner)	$10^{4.7}$
3249	Spain	1992	Abortions	Serum (sow)	$10^{4.7}$
3256	Spain	2005	Respiratory (weaners)	Lung (weaner)	$10^{4.7}$
3262	Spain	2005	Respiratory (weaners)	Lung (weaner)	$10^{5.9}$
3266	Germany	1992	Abortions	Serum (sow)	$10^{7.5}$
3267	Portugal	2006	None (boar)	Serum (boar)	$10^{7.3}$

$TCID_{50}$ = tissue culture infectious dose 50%.
* PAM = porcine alveolar macrophages.

type-I PRRSV isolates is high (Forsberg et al., 2002; Mateu et al., 2003; Stadejek et al., 2002) and at least three or four subtypes are being recognized (Stadejek et al., 2002, 2006, 2008).

PRRSV genome is organized in 9 open reading frames (ORFs). ORF1a and 1b encode the viral replicase as well as other non structural proteins (nsp) (Snijder and Meulenberg, 1998). Of these nsp, nsp2 is the largest and has been shown to contain a large cluster of B-epitopes (Oleksiewicz et al., 2001). Recently, nsp2 and nsp1 of genotype II strains have been reported to be involved in the interplay between the virus and the host responses (Beura et al., 2010; Chen et al., 2010; Subramaniam et al., 2010) by down-regulating mediation of interferons and other cytokines. ORFs 2a, 2b and 3–7 encode for the viral structural proteins. Most of the abovementioned studies about genetic diversity of PRRSV have been developed by examining ORF5 or ORF7 but also some papers focused on ORF3 which is thought to be a molecular clock for PRRSV evolution (Forsberg et al., 2001). Surprisingly, a GenBank search for full length genome – or almost complete genome – PRRSV sequences of genotype I revealed the scarcity of these data and less than 10 full sequences could be found. The aim of the present study was to produce and analyze the sequence of ORFs 1a–7 of contemporary PRRSV field strains of genotype-I for determining within genome diversity and phylogenetic relatedness and to examine some of the immunobiological properties associated with those strains.

2. Materials and methods

2.1. Strains and sequencing

Six field PRRSV strains (2982, 3249, 3256, 3262, 3266 and, 3267) were randomly selected among non epidemiological related strains. Additionally, the vaccine strain Porcilis PRRS was also sequenced. Origin and characteristics of these strains are shown in Table 1. PRRSV strains were grown in porcine alveolar macrophages (PAM) obtained from high health pigs of a farm historically free from all major pig diseases including pseudorabies, classical swine fever, PRRS and influenza. For assessing purity of the viral production, PAM batches were tested by reverse transcription (RT)-PCR or PCR for PRRSV, hepatitis E virus, Torque tenovirus and porcine circovirus type 2 according to previously published protocols (Martín et al., 2007; Mateu et al., 2003; Quintana et al., 2002; Segalés et al., 2009). Viral batches were tested for all those pathogens as well. All viral isolates were used as PAM supernatants (passage $n = 3$). Viral RNA was extracted from supernatants by using the QiaAMP viral RNA minikit (Qiagen, Barcelona, Spain) according to the manufacturer instructions. Reverse transcription was done using Superscript II Reverse transcriptase and random hexamers (Invitrogen, Barcelona, Spain). The obtained cDNA was then used in specific PCRs designed to amplify overlapping segments of the viral genome (Table 2). Both strands of PCR products were sequenced using the Genetic Analyzer 3130 XL (Applied Biosystems). When deletions in the genome were found, confirmation was carried out by re-sequencing of the products and synthesizing of new primers flanking the supposed deletion and further amplification and sequencing.

2.2. Analysis of viral sequences

Sequences were examined and purged of errors using Chromas Pro 1 (Larkin et al., 2007). Predicted amino acid sequences were obtained by translation using BioEdit (Hall, 1999). Nucleotide sequences were aligned using Clustal X2 with correction for multiple substitutions. Bootstrap values were calculated after 1000 iterations using the Neighbor-joining method. For comparative purposes available full length sequences of genotype I (GenBank accession numbers: M96262-Lelystad virus; AY366525-North American EuroPRRSV; AY588319-LV-4.2.1; DQ864705-strain 01CB1 from Thailand; DQ489311-viral clone; EU076704-strain HKEU16 from Hong Kong; FJ349261-strain KNU07 from Korea and GQ461593-strain SHE from China) and genotype II (GenBank accession numbers: AF066183, AF325691, AY032626, AY424271, AY545985, EU880437, EU708726, Q857656, NC_001961, U87392) PRRSV sequences were also included. Synonymous (dS) and non-synonymous (dNS) substitutions in each ORF were calculated using SNAP (Körber, 2000) available at http://hcv.lanl.gov/content/sequence/SNAP/SNAP.html and expressed as dS – dNS values. Topological trees were built up with Mega4 (Tamura et al., 2007) and rooted on mid-points. Predicted amino acid sequences were also examined for variations in the linear B-epitopes reported by Oleksiewicz et al. (2000, 2001), and in the neutralization epitopes (NE) known to be located in GP4 (Meulenberg et al., 1997) and GP5 (Plagemann, 2004).