IN VITRO CHRARACTERISTICS AND IN VIVO BEHAVIOUR OF A CHIMERIC EQUINE ARTERITIS VIRUS DERIVED FROM AN AVIRULENT STRAIN

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1. Introduction

Equine arteritis virus (EAV) is an enveloped positive-stranded RNA virus, belonging to the *Arteriviridae* family. The viral genome consists of a positive-stranded polyadenylated RNA of 12.7 Kb in size. The 5' three-quarters of the genome contain two large open reading frames (ORFs), ORF1a and ORF1b, which are expressed into two large polyproteins. These polyproteins are post-translationally processed by three virus-encoded proteases into twelve non structural proteins (Snijder *et al*, 1994). The last quarter of the viral genome is occupied by 7 partially overlapping ORFs (2a, 2b and 3 through to 7), which are expressed from a set of subgenomic mRNAs into the structural proteins of the virus (de Vries *et al*, 1992).

The virus induces a disease characterised by fever, apathy, loss of appetite, nasal discharge, conjunctivitis, oedema of the palpebrae, the limbs and the genitals, abortion in pregnant mares and persistent infection of the reproductive tract in post-pubertal stallions (Timoney & McCollum, 1998). Most isolates of EAV induce a sub-clinical or mild infection, however isolates varying in degree of virulence are also found, ex: abortigenic strains. As an approach to identify determinants of virulence and, conversely, attenuation in EAV, we investigated a number of characteristics in virulent and avirulent strains of EAV.

2. Materials and Methods

Construction of the chimeric clone: The backbone of the EAV infectious cDNA clone Leiden clone was used to construct a chimeric clone of an avirulent strain of EAV derived by several passages in vero cells and termed VA variant. A fragment from nucleotide 9643 to 12704, encompassing all the genes encoding the structural proteins of the VA virus was cloned in a shuttle vector. This subclone was used to replace the corresponding fragment in the Leiden clone. RNA transcribed *in vitro* from the chimeric clone was transfected into BHK-21 cells. The rescued virus was assayed *in vitro* and *in vivo.*

Experimental infection: Three ponies were intranasally inoculated with $1x10^{-7}$ PFU/horse of the chimeric virus and one pony was used as control. Clinical and post-mortem specimens were analysed by virus isolation and viral RNA detection. Sera collected throughout the experimental period were tested for EAV neutralising antibodies.

3. Results

An infectious virus was recovered upon transfection of BHK-21 cells with the transcribed RNA. The virus induced cytopathic effect (CPE) within 48 hours p.i., produced small plaques on monolayers of equine lung embryonic cells and had a titre of $1x10^{-7}$PFU/ml.

Sequence analysis showed that the chimeric clone and the rescued virus had a deletion of six nucleotides resulting in loss of amino acids 52 and 53 in the ectodomain of the major viral glycoprotein GP5. This deletion is characteristic of the parent VA virus, showing the chimeric nature of the clone and the rescued virus. However, when compared with the parent VA virus, the clone and the rescued virus showed nucleotide substitutions in ORFs 2a, 2b, 4, 5 and mostly in ORF6.

The clinical signs in ponies inoculated with this chimeric virus were mild. The ponies had slight serous nasal discharge for approximately four days, starting from day 2 p.i.

Virus was recovered from nasal swabs, plasma and from serum samples. Viral RNA was detected from nasal swabs, leukocytes and rectal swabs. Antibody responses to EAV were detected in the inoculated ponies by 9 days p.i.

4. Discussion

Strains of EAV occurring in nature vary in virulence. Consequently, they may induce either asymptomatic infections or clinical signs ranging from mild to severe. Such differences in virulence were evident from *in vivo* studies with biologically cloned variants of EAV (Westcott et al 2001), which also exhibited sequence differences among them. Infection with the parent VA variant resulted in asymptomatic infection, with virus replication limited to the site of inoculation. Unlike the parent VA variant that is avirulent, the chimeric virus was able to produce mild clinical signs. The *in vivo* behavior of the chimeric virus appeared to be intermediate between the parent VA variant and the Leiden clone-derived virus, which is of moderate virulence (Balasuriya et al, 1999). These results suggest that the phenotypic characteristics of the chimeric virus could have been "aquired" from the Leiden part of the recombinant, which comprises the genes yielding the non structural proteins Conversely, it is also conceivable that the nucleotide substitutions in the gene encoding the structural proteins of the VA component of the chimera could have contributed to alter virus properties and thus its *in vivo* behavior. The deletion of six nucleotides, found in the VA variant and in the chimeric virus is also present in other strains attenuated by culturing in Vero cells. Since the recombinant virus was not rendered avirulent, it appears that this deletion *per se* does not have effect in virus attenuation.

5. Conclusion

Properties such as virulence and attenuation of EAV may be specified at the genome level. Further studies with full-length clones of virulent and avirulent strains of the virus will be determinant to reveal the involved factors.

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7. References

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