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Generation of a molecular clone of an attenuated lentivirus, a first step in understanding cytopathogenicity and virulence

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ABSTRACT

Small ruminant lentiviruses infect goats and sheep, inducing clinical disease in a minority of infected animals. Following an eradication campaign, clinical cases may disappear in a population. The complete elimination of these lentiviruses is however difficult to achieve and the spreading of less virulent strains often parallels the elimination of their virulent counterparts. Here, we characterized three such strains isolated from a flock in the post-eradication phase. We completely sequenced their genomes, showing that one of the isolates was most probably the product of a recombination event between the other two viruses. By comparing the sequences of these isolates with those of virulent strains, we found evidence that particular LTR mutations may explain their attenuated phenotype. Finally, we constructed an infectious molecular clone representative of these viruses, analyzing its replication characteristics in different target cells. This clone will permit us to explore the molecular correlates of cytopathogenicity and virulence.

Introduction

Small ruminant lentiviruses (SRLV) are a complex phylogenetic group of retroviruses, comprising of the circulating strains of caprine arthritis encephalitis virus (CAEV) and Visna/maedi virus (VMV), which induce persistent infections in goats and sheep. Infected animals may develop pathological manifestations such as arthritis, pneumonia, encephalitis and mastitis. The incidence and severity depend on two principal factors, the genetic background of the infected animals and the virulence of the infecting SRLV strains. The genetic background of the animals appears to influence the susceptibility to infection and has an important impact on the frequency and severity of the induced lesions (Heaton et al., 2012; Larruskain and Jugo, 2013; White et al., 2014; Ruff and Lazary, 1988; Dolf and Ruff, 1994). The virulence of SRLV is highly variable, encompassing few, highly virulent strains and a majority of strains with low or no apparent virulence (Gudmundsson et al., 2005; Oskarsson et al., 2007; Reina et al., 2009; Angelopoulou et al., 2008, 2006; Barros et al., 2004; Glaria et al., 2012).

The principal target cells are monocytes and macrophages (Narayan et al., 1982). Furthermore, dendritic cells are important target cells *in vivo* (Ryan et al., 2000). In monocytes, virus replication is restricted and productive replication starts after the maturation of monocytes to macrophages (Narayan et al., 1983).

SRLV are phylogenetically divided into 5 groups A–E, which comprise different subtypes, based on their *gag* and *pol* sequences (Shah et al., 2004a). The majority of these subtypes are able to cross the species barrier between goats and sheep, and vice-versa, under field conditions (Shah et al., 2004b; Pisoni et al., 2005; Bertoni and Blacklaws, 2010).

In Switzerland, an eradication campaign began in the 1980s which focused exclusively on goats. This campaign became mandatory in 1998, and resulted in a drastic reduction in seroprevalence (from 60–80% to less than 1%). Simultaneously, the clinical cases of arthritis completely disappeared.

Nowadays, in spite of the success of this campaign, seroconversions still occur on regularly and SRLV has been isolated from different goats and sheep flocks (Cardinaux et al., 2013; Deubelbeiss et al., 2014). The main circulating viruses belong to

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the subtypes A4 and A3, and appear to be of low virulence, with the exception of the mammary gland, where histopathological lesions were detected in adult infected goats (Deubelbeiss et al., 2014).

In this work, we focused on three particular SRLV A4 field isolates, co-circulating in a previously described flock of goats and sheep (Cardinaux et al., 2013). We aimed to sequence the complete genomes of these viruses, permitting us to characterize these particular isolates and to construct a replication competent molecular clone.

Results

Sequencing

In a previous study, we described the phylogenetic characteristics and *in vitro* replication properties of 3 SRLV isolates, obtained from the milk cells of one goat and the PBMC of two sheep living in a mixed flock. In this work, we aimed to sequence the complete genome of the three isolates. Since the 5′ and 3′ ends of the genomes were unknown, we first used two primers (Table 1, primer LTR-F and LTR-R) located in the *env* region and in the LTR, to amplify and sequence the U3 part of the 3′ LTR. Both LTR being identical, we could then determine the 5′ end of the genome.

The complete consensus sequences of g6221, s7385 and s7631 were determined by amplifying and sequencing five overlapping PCR products, using different primers (Table 1).

The three isolates have a total length of 9464 bp; all open reading frames (ORF) corresponding to the main and accessory genes were present and intact (GenBank accession numbers: KT453988 for g6221, KT453989 for s7385 and KT453990 for s7631). Additionally, the derived amino acid sequence of the dUTPase encoding region showed an intact active site compared to the phylogenetically distant equine infectious anemia virus (EIAV) or feline immunodeficiency (FIV) dUTPase proteins (Fig. 1s Supplementary material). The dUTPase inactivating E to G mutation described by Turelli et al. (1996) at position 109 in the CAEV-CO strain was intact (E) in our molecular clone.

Phylogenetic analysis and comparison between the 3 isolates

The 3 isolates could be classified as SRLV subtype A4, based on phylogenetic analyses performed with the entire sequences (Fig. 2) or the *gag*, *pol* and *env* genes, as well as the LTR (data not shown).

A pairwise DNA distance analysis over the complete genome (SimPlot; Lole et al., 1999), shown in Fig. 1, confirmed the phylogenetic analysis and revealed that the g6221 was much closely related to a previously sequenced SRLV-A4 isolate (GenBank: AY445885) than to the prototypic sequences CAEV-CO (SRLV-B1 genotype, GenBank: NC_001463.1) and VMV 1514 (SRLV-A1 genotype, GenBank: M60610.1). As expected, the env gene showed the highest divergence between the g6221 virus and the reference strains, along with the LTR sequence, which was quite distant to its counterparts in the prototypic strains (Fig. 1).

Recombination event

Using the RDA-4 software, we demonstrated that isolate s7385 was most probably the product of a recombination event between the two parental viruses g6221 and s7631 (Martin et al., 2015). As shown in Fig. 3, the recombination event was located in the highly variable region encompassing the V4-V5 domains of env, between positions 7753 and 8311 of the alignment. The probability figures, using different methods, were 3.156×10^{-03} for RDP, 3.083×10^{-04} for MaxChi and 2.233×10^{-03} for Chimaera.

LTR analysis

The LTR sequences of the three viruses were highly similar to each other but, as shown in Fig. 1, quite distant to the prototypic SRLV-A and -B sequences. Different transcription factor binding sites were mapped in the long terminal repeat, such as 5 AP-1 sites, one AP-4, 2 AML(vis) and an E-box (Fig. 4).

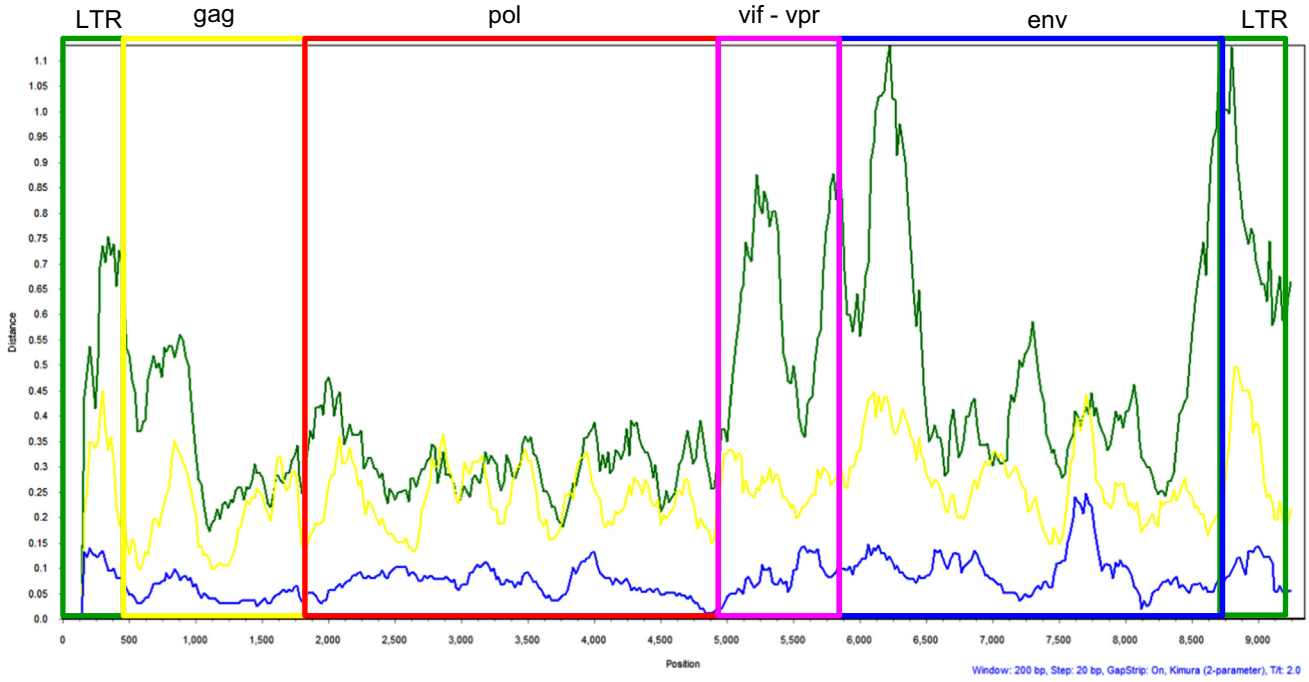

Fig. 1. A Pairwise DNA Distance analysis of the complete genomes (SimPlot) was performed using the SimPlot software. Strains AY445885 (blue line), CAEV-CO (green line) and MVV 1514 (yellow line) were compared to the SRLV-A4 isolate g6221. The two long terminal repeats (LTR) and the genes gag, pol, vif, vpr (former tat) and env are shown on top.