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Short communication

Seroconversion against SU5 derived synthetic peptides in sheep experimentally infected with different SRLV genotypes

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ABSTRACT

Synthetic peptides were generated, corresponding to SU5 domain of envelope glycoprotein of Italian SRLV isolates It-561 and It-Pi1, belonging respectively to MVV- and CAEV-like genotypes. The peptides, encompassing an N-terminal variable and a C-terminal conserved antibody-binding site, were used in an ELISA assay to analyse the sera of two groups of sheep experimentally infected with these isolates. The kinetics and specificity of the humoral response to the homologous and heterologous antigen and the affinity maturation of the sera were evaluated. Seroconversion occurred between week 3 and 8. The response to SU5 antigen was mostly type-specific. The few broadly reacting sera may reflect the production of antibodies directed to the SU5 constant antibody-binding site. All sera underwent with time avidity maturation, resulting in the appearance of high affinity antibodies. This study suggests constant monitoring of the circulating viral variants to develop a panel of diagnostic peptides representative of local genotypes.

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1. Introduction

Maedi Visna Virus (MVV) and Caprine Arthritis Encephalitis Virus (CAEV) are non-oncogenic lentiviruses of the Retroviridae family, presently referred to as Small Ruminant Lentiviruses (SRLVs) (Zanoni, 1998). A recent phylogenetic classification of these viruses defines two main groups, A and B, including respectively MVV-like and CAEV-like genotypes (Shah et al., 2004). SRLV infections occur almost worldwide and cause relevant economic losses. Therapy and effective vaccine are not yet available (Gonzalez et al., 2005; Petursson et al., 2005; Torsteinsdottir et al., 2007; Niesalla et al., 2009; Reina et al., 2008), therefore eradication and prevention of the infection largely depend on early, efficient and correct identification of infected animals (de Andrés et al., 2005). This is routinely done by serological analysis, the ELISA assay being the most sensitive and suitable technique both for large-scale screening and individual examination. Several ELISA protocols have been developed so far, mostly based on antigens from a single viral strain. The specificity of these assays is generally high, but the sensitivity shows extensive variability (de Andrés et al., 2005) due to antigenic heterogeneity of SRLVs. Recent analysis of two Italian SRLV isolates, MVV-like It-561 and CAEV-like It-Pi1, demonstrated that gag-encoded capsid (CA) and matrix (MA) proteins carry type-specific epitopes and most sheep and goat sera reacted to these antigens in a type-specific manner, irrespective of the species of origin (Grego et al., 2002, 2005). Furthermore, homologous CA-MA fusion protein was able to detect seroconversion at an earlier stage compared to the heterologous protein (Lacerenza

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et al., 2006). The present study extends the analysis of the humoral response induced by It-561 and It-Pi1 to SU5, one of the major epitopes of the envelope (Env) protein (Bertoni et al., 2000; Valas et al., 2000). Two SU5 synthetic peptides were generated, corresponding to It-561 and It-Pi1 sequences. The peptides were used as antigens to assay sera from two groups of sheep experimentally infected with these viruses, in order to investigate the kinetics and specificity of the humoral response to the homologous and heterologous antigen.

2. Materials and methods

2.1. Viral strains and experimental infection

SRLV strains It-561 (genotype A) and It-Pi1 (genotype B) (Grego et al., 2002) were used in the present study. Infection of sheep with these strains has been described by Lacerenza et al. (2006). Detection of viral sequences in blood of experimental sheep was done by *LTR* PCR (Extramiana et al., 2002) and *Pol* PCR (Grego et al., 2002).

2.2. Cloning, sequencing and sequence analysis of env region coding for SU5

An *env* gene fragment encompassing the 75 bp "SU5 total" domain (Mordasini et al., 2006) coding sequence (nt 7800–7874) was amplified from DNA of foetal ovine lung fibroblasts infected respectively with It-561 and It-Pi1 viral stocks. Primer sequences (Bertoni et al., 2000) were as follows: 563F: GAYATGRYRGARCAYATGAC (nt. 7272–7291); 567F: GGIACIAAIACWAATTGGAC (nt. 7482–7501); 564R:GCYAYATGCTGIACCATGGCATA (nt. 8089–8067). The nucleotide positions refer to the CAEV sequence published by Saltarelli et al. (1990) (Genbank accession number M33677). Amplified fragments were cloned and sequenced. Genetic distances were calculated with MEGA 3 (Kumar et al., 2001) and used to construct a neighbor-joining tree with the Tamura-Nei two-parameter distance option (Tamura and Nei, 1993).

2.3. Peptide ELISA

Peptides corresponding to the 25aa "SU5 total" domain of both viruses, were resuspended in 0.1 M carbonate buffer pH 9.6 at 5 μg/mL. ELISA plates were coated overnight at 37 °C (50 μL/well), incubated for 1 h at 37 °C with sera diluted 1/20 and for 1 h at room temperature with a 1:8000 dilution of peroxidase-labelled anti-sheep/goat IgG monoclonal antibody (Sigma). Optical density was measured at 405 nm wavelength after 40 min incubation with ABTS (Sigma).

2.4. Cut-off

A panel of sera collected before experimental infection as well as sequential sera from two mock-infected sheep, were repeatedly assayed: no significant interplate variation of the absorbance was observed. The cut-off was calculated as mean absorbance of negative sera + 3 × S.D. Samples with absorbance higher than 0.3 were considered positive with a 99% confidence level. A serum collected from a sheep belonging to a long term seronegative flock was loaded on each plate.

2.5. Antibody avidity measurements

The avidity index values of SU5-specific antibodies were measured by testing the stability of the antigen–antibody complexes following a wash in 8 M urea (Mordasini et al., 2006). Antibodies with avidity indexes <30% were considered to be of low affinity; those with values between 30% and 50% of intermediate avidity and those with values >50% of high avidity.

3. Results

3.1. Cloning and sequencing of SU5-encoding env region

The *env* sequences encompassing respectively the SU5 domain of It-561 and It-Pi1 strains (Genbank accession numbers: It-561: EU702487: It-Pi1: EU709743) were aligned with MVV and CAEV prototypic *env* sequences of worldwide origin. It-561 clusters with representatives of group A genotypes and It-Pi1 with B genotypes (Fig. 1a), in agreement with the results of a phylogenetic analysis of *gag* and *pol* sequences (Grego et al., 2002, 2005). The SU5 aminoacid sequences of It-561 and It-Pi1 share homology in the N-terminal region, which is well conserved among MVV and CAEV strains, and diverge in the C-terminal, more variable portion (Fig. 1b).

3.2. Peptide ELISA

Peptide ELISA assays were performed on a panel of sera obtained over a period of up to 92 weeks p.i. from three groups of experimentally infected sheep: group A, sheep 1–5, infected with It-561; group B, sheep 6–9, infected with It-Pi1 (Fig. 2a and b) and group C, four mock-infected sheep. Group C sheep remained seronegative throughout the study (data not shown). Group A sheep 1–4 seroconverted to the homologous antigen between week 4 and 8 and remained positive throughout the study. Sheep 1–3 were completely negative to the heterologous It-Pi1 peptide, while sheep 4 raised a weak humoral response to It-Pi1, from week 77 onwards. Sheep 5 did not seroconvert and was the only one negative to both *LTR* and *Pol* PCR assays performed throughout the study to confirm the presence of virus in the infected animals (data not shown). Group B sheep seroconverted to the homologous antigen between week 3 and 8; all sheep remained positive until the end of the experiment, with high absorbance values. Only sheep 9 responded to the heterologous antigen from week 8 onwards. The absorbance, which was about twofold to threefold lower than for the homologous antigen, reached a peak at week 22 and gradually decreased thereafter, to values just above the cut-off.

3.3. Kinetics of antibody avidity maturation

The avidity maturation of sera was assayed at week 8 p.i., corresponding to the first antibody peak, at week 35

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