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.

Keywords Small ruminant lentiviruses; Envelope glycoprotein; SU5 peptides; Type-specific seroconversion

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 and 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 and Grego et al., 2005). Furthermore, homologous CA-MA fusion protein was able to detect seroconversion at an earlier stage compared to the heterologous protein (Lacerenza 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 and 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.

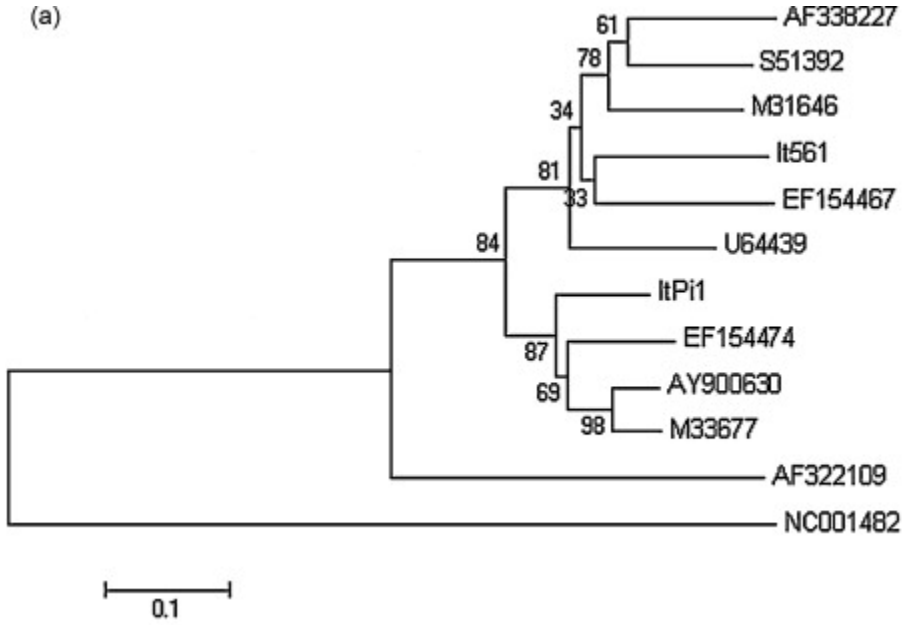
2.5. Antibody avidity measurements

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 and Grego et al., 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).



(b)

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                ....|....| ....|....| ....|
                    5         15         25
It-561          KVRAYTYGVV EMPSNYEQKD RKKRD
EF154467        R..T..... D..T..VELQ .GR.K
U64439          .......... ...KS.MESQ KR.KR
S51392          R.......E. D..QS.LE.N .RNAF
M31646          .......... D..KA.RE.N MRNKR
AF338227        .......... ...QS.MEER GEN.R
It-Pi1          R........I D..K...KTN LNRKK
EF154474        R.......MI .L.K...KIS LNR.K
M33677          .........I ...E..AKTR IIN.K
AY900630        .........I ...E..AKTR IIN.R
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Fig. 1.

(a) Phylogenetic analysis of It-561 and It-Pi1 env fragments encompassing SU5 coding region. The neighbor-joining tree was constructed using MEGA. Bootstrap values are based on 5000 repetitions. The accession numbers refer to the following strains: AF338227 (MVV K1514, Iceland); S51392 (MVV EV1, Great Britain); M31646 (SA-OMVV, South Africa); EF154467 (SRLV 12-5, Italy); U64439 (OLV 85/34, USA); EF154474 (SRLV 12–20, Italy); AY900630 (CAEV Gansu, China); M33677 (CAEV-Co, USA), AF322109 (CAEV 1GA, Norway); NC001482 (FIV). (b) Alignment of SU5 aminoacid sequences of It-561 and It-Pi1 with MVV and CAEV strains.

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.

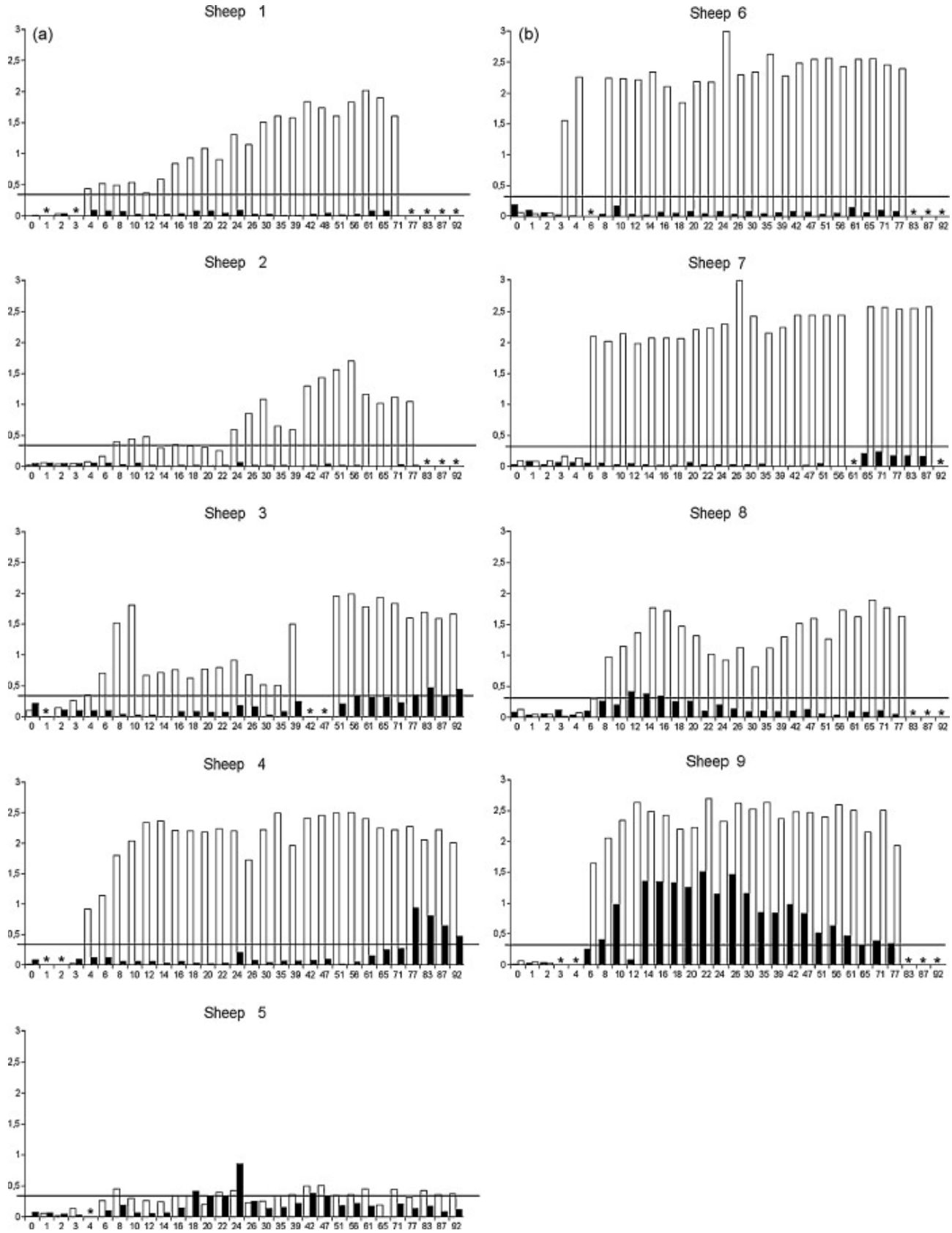

Fig. 2.

Kinetics of humoral responses of experimentally infected sheep to homologous (white bars) and heterologous (black bars) antigen. The cut-off was set at 0.3 OD. *Not available. (a) It-561 infected sheep. (b) It-Pi1 infected sheep.

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 and at week 78. The results obtained for group A sheep are depicted in Fig. 3a. Sheep 2, 3 and 4 underwent a marked increase of antibody avidity over the period analyzed. All avidity indexes of sheep 1 were low; therefore additional time points were analyzed. Maturation to high avidity was detected at week 56, followed by a gradual decrease to low avidity by the end of the experiment (data not shown. Group B sheep underwent a marked avidity maturation, reaching the highest values at week 35 p.i. (Fig. 3b).

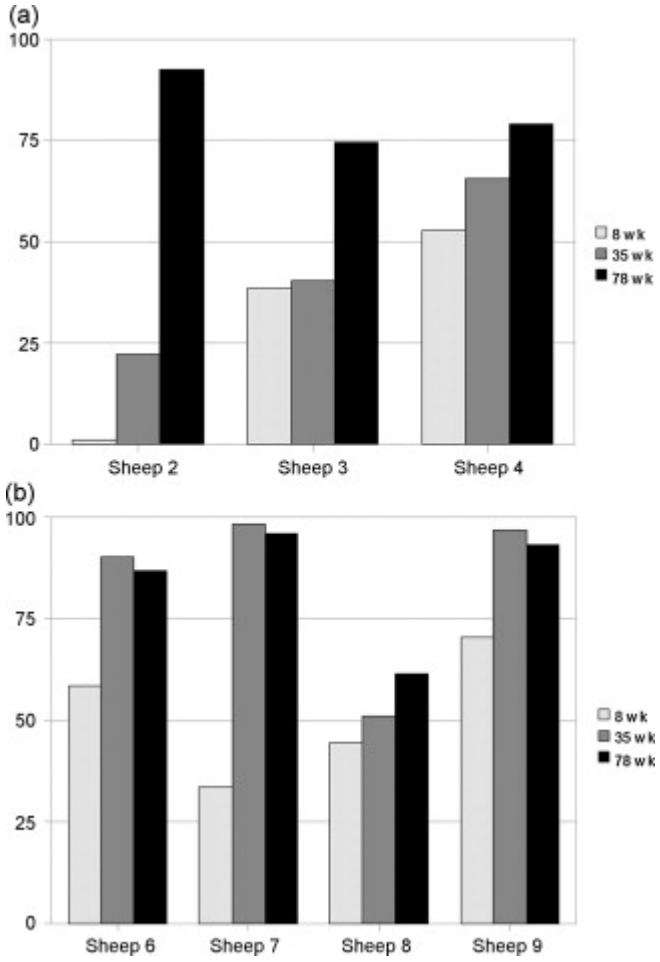

Fig. 3.

Avidity maturation of anti-SU5 antibody taken at 8, 35, 78 weeks p.i. (a) Sheep infected with It-561; (b) sheep infected with It-Pi1. Serum antibodies with avidity index values lower than 30% are designated low avidity antibody, those with avidity index values between 30% and 50% are intermediate-avidity antibodies, and those with values higher than 50% are considered high avidity antibodies.

4. Discussion

Most ovine isolates collected in Italy belong to the CAEV-like group (Grego et al., 2002) and co-infection of goats with both MVV- and CAEV-like strains in an Italian flock has recently been reported (Pisoni et al., 2007). In the last years serological assays based on Gag antigens derived from Italian isolates belonging to both phylogenetic groups were developed and applied in field conditions: the response obtained was largely type-specific, indicating the opportunity to use antigens representative of both genotypes to improve the sensitivity of the diagnostic assays (Grego et al., 2002 and Grego et al., 2005). Furthermore, when sequential sera collected from sheep experimentally infected with either It-561 or with It-Pi1, were analyzed by ELISA with both homologous and heterologous CA-MA fusion proteins, the homologous

antigen detected infected animals up to months before the heterologous antigen (Lacerenza et al., 2006). In this study the same panel of sequential sera was analyzed with an Env ELISA based on It-561 and It-Pi1 SU5 peptides. Five epitopes have been identified in the SU subunit of Env (Bertoni et al., 2000 and Valas et al., 2000). SU5 in particular, a 25 aminoacid sequence located at the C terminus of SU, is immunodominant and partly type-specific: it carries at least three antibody-binding sites, one in the N-terminal conserved region, one within the C-terminal most variable domain, and the third encompassing the junction between the variable and conserved regions (Mordasini et al., 2006). In this study, two env gene fragments encoding respectively It-561 and It-Pi1 SU5, were sequenced. Alignment of these fragments with sequences of MVV and CAEV prototypic strains of worldwide origin showed that It-561 clusters with the MVV strains and It-Pi1 is phylogenetically related to classical CAEV-like prototypes. The SU5 N-terminal amino acid sequences of the two strains share extensive homology; the C-terminal region is more divergent, confirming the results of Mordasini et al. (2006). Seroconversion kinetics of groups A and B sheep were similar, with antibodies being mostly type-specific. The few broadly reacting sera may reflect the production of antibodies directed to the constant antibody-binding site. The antibody levels varied between animals and for the same animal during the time course of the experiment. In group A, the first antibody peak was followed by a slight decrease of reactivity and then by a rise to three to fourfold higher values. One sheep of this group did not seroconvert. This result was most likely caused by a technical failure during the experimental infection, since the sheep was repeatedly negative to both LTR and pol PCR assays which were performed on blood samples of the experimental animals to confirm the presence of viral genomes. Group B sheep developed immediately an antibody response well over the cut-off. All sera underwent with time avidity maturation, resulting in the appearance of high affinity antibodies, although with different kinetics. It-Pi1 sera, which developed higher antibody levels from the beginning, underwent high avidity maturation at an earlier time point than It-561 sera. Sheep 1 showed a peculiar pattern of avidity maturation, with indexes decreasing from week 56 onwards, to return to low values by the end of the experiment, in spite of a persisting high antibody level. The reason for sheep 1 behaviour is unclear; the genetic variability of SU5 could possibly explain the disappearance of the high affinity antibody-producing clone(s). Alternatively, this sheep may present some failure in the mechanisms that underly the ability to maintain the production of high affinity antibodies. A failure of T helper function may be responsible for the loss of high affinity antibodies as well as the associated decay of the antibody titer, that have been reported in individuals who later developed AIDS (Chargelegue et al., 1993, Chargelegue et al., 1995 and Thomas et al., 1996). SRLV infection, however, does not involve the lymphocyte compartment, and sheep 1 antibody titer does not decrease, therefore a different mechanism may be responsible. A comparison of the results obtained in this study and in Lacerenza et al. (2006) shows that both CA-MA fusion antigens and SU5 peptides detect infection at early stages, from week three onwards. Furthermore, while It-561 drives type-specific antibody response towards both Gag and Env antigens, It-Pi1 seems to drive production of type-specific antibody only against SU5. In fact, all group B sheep reacted against the heterologous Gag subunits as well (Lacerenza et al., 2006). Since the humoral response to SU5 is mainly directed towards the variable antibody-binding site, a constant monitoring of the circulating viral variants will be necessary to develop a panel of SU5 peptides representative of most local genotypes.

Conflict of interest statement

All authors deny any financial and personal relationships with other people or organizations that could inappropriately influence this work.

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