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Maedi-visna virus persistence: Antigenic variation and latency

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ABSTRACT

Maedi-visna virus (MVV), a lentivirus of sheep, shares with other lentiviruses the ability to establish a lifelong infection. In this study five sheep were infected intravenously with MVV and housed together with a number of uninfected sheep for natural transmission. All virus isolates from ten sheep that had been infected naturally had multiple mutations in the principal neutralization domain in Env and were antigenic variants, while three of four isolates from the carrier sheep had identical sequences to the infecting strain and were not antigenic variants. There was evidence of positive selection in the gene, particularly in amino acids comprising the neutralization epitope and some adjacent glycosylation sites. Together these results suggest that virus persistence is acquired by a reservoir of latent viruses, and that there is selection for antigenic variants of virus that is transmitted naturally.

1. Introduction

Maedi–visna virus (MVV) is a macrophage-tropic lentivirus of sheep causing slowly progressive interstitial pneumonia (maedi) and demyelinating encephalomyelitis (visna) [1]. The virus infects monocytes, but productive virus replication only takes place upon differentiation of the monocytes into macrophages [2–5]. Maedi–visna virus (MVV) establishes a lifelong infection in spite of strong immune responses in the host, both humoral and cell mediated [6–10]. We originally proposed that one way for the virus to persist in the face of a strong antibody response may be through antigenic drift [11]. A number of subsequent studies indicated progressive antigenic variation in single infected animals [12–16], and we have indeed shown that the humoral immune response is effective at selecting antigenic variants of MVV in vivo [17]. However, results from long term infection experiments with MVV indicated that although antigenic variants were found at a high frequency, they could not be the sole cause of virus persistence since the majority of virus isolates within an infected animal up to seven years after infection were not antigenic variants [18,19].

Several lines of evidence have been obtained for selection of antigenic escape mutants by neutralizing antibodies in other lentiviral infections, notably EIAV, SIV and HIV infection [20–24]. The lentiviral Env proteins are among the most heavily glycosylated proteins known with carbohydrates comprising more than 50% of the molecular weight [25] and it has been suggested that the role of this extensive glycosylation is to shield the virus from neutralizing antibodies. Antibody escape mutants of HIV and SIV contain more mutations in N-linked glycosylation sites than can be expected by chance [22,26], and this led Wei et al. to postulate an evolving glycan shield mechanism of neutralization escape where selected changes in glycan packing prevent neutralizing antibody (Nab) binding but not receptor binding [22]. Indeed, we have shown that changes in a potential N-linked glycosylation site (PNG) in the principal neutralization domain (PND) of MVV gp135 can result in antibody escape without affecting replication rate [27].

In this study we analyzed virus isolates from sheep that had been housed together with sheep experimentally infected with MVV for natural transmission. The main route of transmission of MVV is by aerosol between animals over distances of up to several meters, and by colostrum and milk [28,29]. Part of the *env* gene comprising the principal neutralization domain (PND) of MVV [30] was sequenced and the neutralization phenotype of the isolates was determined.

2. Material and methods

2.1. Virus and cells

The MVV strain K796 is a virus isolate from a transmission experiment where virus was passed from sheep to sheep [1,30]. Virus was propagated in monolayers of sheep choroid plexus (SCP) cells. SCP cells

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were established as described previously [31] and grown in Dulbeccós modified Eagles medium (DMEM) supplemented with 200 units/ml penicillin, 100 units/ml streptomycin, 2 mM glutamine and either 10% or 1% lamb serum, for growth medium or maintenance medium respectively.

2.2. Experimental animals and infection

In this study, five rams were heavily infected intrapulmonarily with MVV strain K796 (5×10^7 $TCID_{50}$) to serve as infected contacts (donors) in natural transmission studies. These animals were mixed with sheep of Icelandic breed, males and females, which had been vaccinated with formalin inactivated K796 virus using alum as an adjuvant, as well as unvaccinated control sheep. All the vaccinated sheep developed neutralizing antibodies before being mixed with the infected sheep [32].

Virus isolates and sequence information were obtained from 4 of the donor animals and 10 of the in-contact animals (4 vaccinated and 6 controls).

2.3. Virus isolation

Virus was isolated at sacrifice from lungs, spleen, lymph nodes or blood of the sheep by explants and coculture with sheep choroid plexus (SCP) cells as described previously [31,33].

2.4. Neutralization assay

Neutralization tests were performed by mixing serial twofold dilutions of serum with 100 $TCID_{50}$ of virus. The samples were incubated at room temperature for 24 h and then inoculated in quadruplicate onto monolayers of SCP cells in 96-well plates. Cytopathic effects were monitored microscopically for 2 weeks. Neutralization titer was expressed as the reciprocal of the serum dilution which caused complete neutralization in 50% of inoculated cultures. The K796 – specific antiserum was serum from a sheep that had been infected with the MVV strain K796 and neutralized K796 but not the heterologous MVV strain K1514.

2.5. Infection of cells and lysis for PCR

SCP cells were infected with the different MVV isolates at an m.o.i. of 0.5 $TCID_{50}$/cell. When cytopathic lesions started to appear, usually 2–3 days after infection, the cells were harvested and resuspended in 100 µl lysis buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 0.5% Triton X-100, 0.001%SDS, 300 µg proteinase K/ml) and incubated overnight at 37 °C. After 15 min of inactivation at 96 °C, 3 µl of the lysate were used in a 20 µl PCR reaction.

2.6. PCR, cloning and sequencing of viral DNA

Cell lysates from infected cells were amplified with primers V7499BamHI forward and V7979EcoR1 reverse, 5′-CGCGGATCCATAGCACCATAACAGGAA-3′ and 5′- CGGAATTCTTTGCG ACACCGAGACCA-3′ respectively (nucleotide numbering as published for strain KV1772) [34]. The amplified products were cut with *Bam*HI and *Pst*I and cloned into pUC19 vector, each clone from a separate PCR to ensure that the clones were independent. The inserts were sequenced in both directions using universal primers for the vector.

2.7. Sequence analysis

The sequences were aligned using Clustal Omega [35], and then insertions and deletions adjusted manually in GeneDoc [36]. Recombination was assessed with the RIP software on the HIV sequence database website (http://www.hiv.lanl.gov) [37], focusing on five DNA sequences that branched off deep branches in the tree. These were compared against all unique sequences in the alignment (26 total), using 200 bp window size and treating insertion and deletion variants as full characters. The number of putative glycosylation sites and charges of variable regions was estimated with HIV Lanl tools VAR_reg_char. Statistics summarizing sequence diversity were estimated with DNAsp vs. 5.10 [38] and SNAP (www.hiv.lanl.gov), with three sequences removed from dataset as those programs do not handle missing data.

The evolutionary history was inferred in MEGA6 [39], by Maximum Likelihood method using the Hasegawa-Kishino-Yano model and Gamma distribution of evolutionary rates (5 categories, and G parameter = 0.2653) [40]. The tree with the highest log likelihood (−1501.1850) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. All positions containing gaps and missing data were eliminated. There were a total of 412 positions in the final dataset which included 64 sequences. Recoding insertions and deletions (indels) as characters did not alter the tree topology. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the sequences analyzed [41].

The rate of evolution at each site in the protein was calculated and tests of positive selection were conducted with the Selecton 2.2 program (http://selecton.bioinfo.tau.ac.il) [42]. We contrasted models M8 (where the Gamma distributed parameter ω can be maximum 1 – allowing for negative selection and neutral sites), and the more complex model of M8a (where ω can take values considerably larger than 1–in order to detect positive selection). We used the default number (8) of distinct values for ω. These nested models were evaluated with Likelihood ratio tests. Distribution of polymorphism, silent, non-synonomous, insertion and deletion (indel) and positively selected (from the Selecton program), within the Env protein was analyzed with $\chi 2$ and Fisher's exact tests in R 3.1.0 [43].

Table 1
Neutralization of virus isolates with type-specific anti-K796 serum.

Virus strain isolate	Source of virus	Time from exposurem (months)	Neutralization titre (K796 antiserum)
K796	inoculum		512
K10s	carrier sheep	120	64
K10lu	carrier sheep	120	64
K11s	carrier sheep	54	< 4
K14s	carrier sheep	78	64
K15s	carrier sheep	78	64
K36s	unvaccinated sheep	48	< 4
K296bl	unvaccinated sheep	48	< 4
K898ly	unvaccinated sheep	48	< 4
K22s	unvaccinated sheep	108	< 4
K22lu	unvaccinated sheep	108	< 4
K418bl	unvaccinated sheep	73	< 4
K486s	unvaccinated sheep	78	< 4
K95s	vaccinated sheep	52	< 4
K417ly	vaccinated sheep	55	< 4
K485s	vaccinated sheep	78	< 4
K485lu	vaccinated sheep	78	< 4
K718ly	vaccinated sheep	48	< 4

Virus was isolated from blood (bl); spleen (s); lymph node (ly); or lung (lu).