Review Article

Maedi-Visna Virus and Caprine Arthritis-Encephalitis Virus: Distinct Species or Quasispecies and its Implications for Laboratory Diagnosis

John Pasick

ABSTRACT

The lentiviruses responsible for causing maedi-visna or ovine progressive pneumonia in sheep and caprine arthritis-encephalitis in goats have long been considered distinct, albeit related, viral species. Evidence, primarily in the form of nucleic acid sequence data, suggests this distinction may not be as absolute as once thought. These lentiviruses might better be viewed in the context of viral quasispecies whose individual members exhibit varying host range and pathogenic capabilities. Implications for diagnostic testing and control of these diseases are discussed.

RÉSUMÉ

Les lentivirus responsables de causer le maedi-visna ou pneumonia progressive ovine chez les moutons et l'arthrite-encéphalite caprine chez les chèvres ont longtemps été considérés comme des espèces virales dinstinctes, quoique reliées. Certaines évidences, principalement au niveau de la séquence des acides nucléiques, suggèrent que cette distinction ne serait pas aussi évidente qu'on pourrait le croire. Dans un contexte de classification, ces lentivirus pourraient être considérés comme des membres de quasi-espèces démontrant des variations au niveau de leur hôte et de leur potentiel pathogène. Les implications pour les épreuves de diagnostic et le contrôle de ces maladies sont discutées.

(Traduit par docteur Serge Messier)

Maedi-visna (MV), also known as ovine progressive pneumonia (OPP), and caprine arthritis-encephalitis (CAE) are lentiviral diseases of sheep and goats. Both diseases exhibit a chronic, progressive course with characteristic mononuclear inflammatory cell infiltration of target organs that include the lungs, the joints, the mammary glands, and the central nervous system. The earliest recognition of MV probably dates back to 1915, when a progressive interstitial pneumonia was reported in South African sheep. A similar syndrome was later reported in Montana sheep in 1923 (1). However, it was not until an epidemic of progressive pneumonia (maedi) and paralysis (visna), involving Icelandic sheep between 1939 and 1952 (2), that this disease received serious attention. The origin of this outbreak was traced to the importation of Karakul sheep from Germany in 1931 (2). The etiologic agent, maedi-visna virus (MVV), was eventually isolated from the lungs of a sheep with progressive interstitial pneumonia in 1967 (3). In contrast to MV, CAE is a more recently recognized syndrome of goats. It was first described in the mid 1970s, in the northwestern United States, following an epizootic of leukoencephalomyelitis accompanied by subclinical interstitial pneumonia in kid goats (5). In retrospect, this outbreak probably did not result from the sudden introduction of a new virus, but from changes in management practices that facilitated spread of a previously existing virus. Caprine arthritis-encephalitis virus (CAEV) was subsequently isolated from the synovial membranes of an adult goat with arthritis from a herd having a high incidence of leukoencephalomyelitis (5). Both diseases are currently recognized as having a worldwide distribution.

A number of isolates of both viruses have been characterized genetically, antigenically, and phenotypically. As early as 1981 ovine and caprine lentiviruses were recognized as having a close kinship based on their immunologic cross-reactivity (6). Later reports confirmed that MVV and CAEV have at least one epitope in common in each of their structural proteins (7–9). Despite evidence showing that MVV and CAEV are closely related antigenically, it was also recognized that both viruses have the capacity to undergo significant antigenic variation within the same infected animal over time (10,11).

Beginning in 1985, sequence information began to emerge on the 9.2-kb genomes of MVV and CAEV. The K1514 strain of MVV, isolated from the Icelandic epidemic, was the first to have its genome entirely sequenced (12,13). This was followed by complete nucleotide sequence information for the South African ovine MVV SA-OMVV (14), the CO strain of CAEV (15), and the British MVV isolate EV1 (16), along with partial sequence data for a number of other isolates (17–22). Based on partial nucleotide sequence information for the *pol* and SU domain of the *env* gene, phylogenetic trees were constructed illustrating the genetic relationships among a number of small ruminant lentivirus (SRLV) isolates (19,21). This analysis resulted in the SRLVs being divided into four genetic groups. Interestingly, the three MVV strains

Canadian Food Inspection Agency, National Centre for Foreign Animal Disease, 1015 Arlington Street, Suite T2300, Winnipeg, Manitoba R3E 3M4. Phone: (204) 789-2013. Fax: (204) 789-2038. e-mail: pasickj@em.agr.ca.

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K1514, SA-OMVV, and EV1, for which complete sequence information is available, were all placed within genetic group IV. Within that group, K1514 and SA-OMVV exhibit 81.5%, 87%, and 80% homology in the deduced amino acid sequences of their *gag, pol*, and *env* proteins, respectively (15). Similar degrees of homology exist between K1514 and EV1 and EV1 and SA-OMVV (16). By comparison, CAEV CO and MVV K1514 exhibit only 74.8%, 77.5%, and 60% homology in the deduced amino acid sequences of their respective *gag, pol*, and *env* proteins (15). Accordingly, CAEV CO was assigned to genetic group II (21) which, so far, is comprised of only two other SRLV isolates of caprine origin, and for which only partial *pol* sequence information is available. SRLV members of genetic groups I and III originate from sheep and goats. Surprisingly, analysis of non-overlapping 303 and 475 nucleotide fragments of the *pol* gene of French SRLVs members of genetic group I, which originated from sheep, revealed greater similarity with CAEV CO than with MVV K1514 (19,21). The deduced amino acid sequence homology of members within this group ranges from 83% to 100% based on the 303-nucleotide *pol* fragment. When group I viruses were compared with CAEV CO and MVV K1514, the deduced amino acid sequence homologies ranged from 87% to 100% and 83% to 88%, respectively. Dutch workers have also compared the DNA sequence of amplified portions of the *gag* and *pol* genes of six isolates of MVV and CAEV of American, Dutch, and Swiss origin, and found closer relationships among these isolates than with K1514 (22). Further analysis of a 420-nucleotide fragment from the highly variable, carboxy terminus of the SU domain of the *env* gene found 21 French SRLVs to be, on average, equally distant from CAEV CO and MVV K1514 (21) with 78% and 79% deduced amino acid sequence homologies, respectively. This finding does not appear to be unique to SRLVs of French origin since two phenotypically distinct North American ovine lentiviruses, 85/34 and 84/28, have also been shown to be genotypically more akin to CAEV than to MVV, on the basis of their *env* gene sequences (20). These findings have led to the suggestion that the French and North American ovine lentiviruses may have originated from CAEV or a CAEV-like virus (19–21). Earlier experimental evidence, demonstrating that sheep can be infected with CAEV (23,24) and goats with MVV (23), supports the possibility of cross-species transmission, although direct evidence that this occurs in nature is lacking.

SRLVs have also been separated on the basis of their biological or phenotypic properties. The designations type I, or MVV-like, and type II, or CAEV-like, have been given to viruses exhibiting specific pathogenic potentials (25,26). Type I viruses tend to be highly cytopathic in vitro, pathogenic in vivo and induce the production of neutralizing antibodies, while type II viruses tend to produce nonlytic and persistent infections in cell culture, exhibit low pathogenicity in infected animals, and induce non-neutralizing antibodies. SRLV genomic sequence information has provided some clues about the nuances that relate genotype to phenotype. As an example, the ovine lentivirus isolates 85/34 and 84/28, which are representative type I and type II SRLVs, respectively, were found to be 97.5% homologous in their env nucleotide sequences (20). Similarly, two infectious molecular clones of MVV, namely KV1772-kv72/67 and LV1-1KS1, each related to K1514, differ from each other in only 1% of their total nucleic acid sequence. They do, however, exhibit sharply contrasting pathogenic potentials when inoculated intracerebrally into sheep. The KV1772-kv72/67 clone was reported to induce pronounced CNS pathology and a strong humoral immune response, while LV1-1KS1 induced little or no CNS pathology or humoral immune response (27). Further work in this area should make it possible to associate specific viral genetic determinants with pathogenicity.

The traditional view that MV and CAE are caused by distinct etiologic agents may be outdated in light of the foregoing discussion. Instead, SRLVs might better be viewed within the conceptual framework of the viral quasispecies. This concept was first proposed by Manfred Eigen and coworkers (28) and is defined as a complex, self-perpetuating population of diverse entities that act as a whole. Experimental evidence and theoretical arguments indicate that group rather than individual selection operates within RNA virus systems, and that entire quasispecies rather than individual viral genomes is the target of selection (29). The fact that RNA viruses in general, and lentiviruses in particular, exist as quasispecies is attributed to the fact that RNA polymerases have intrinsically high error rates and lack proofreading mechanisms (29). The result is that RNA viruses reproduce themselves imperfectly. The error rate of the viral RNA polymerase is directly responsible for determining the size and integrity of the quasispecies; low error rates are associated with quasispecies occupying small volumes of sequence space while high error rates result in quasispecies occupying larger volumes of sequence space.

This variability is not only instrumental in the ability of lentiviruses to evade the host's immune response, and hence produce persistent infections, but it may also enable them to cross species barriers, as may be the case for MVV and CAEV. In fact, the picture beginning to emerge for the SRLVs is not of a collection of distinct species, but of a heterogenous group possessing variable host range and pathogenic capabilities. This view has obvious practical implications for the design of diagnostic tests and implementation of control measures.

Rudimentary evaluation of the polymerase chain reaction (PCR) as a tool for detecting lentiviral infections in small ruminants has been carried out in a small number of laboratories (22,30). Primers based on the published sequences of MVV K1514 (12) and CAEV (31), and designed to amplify the LTR, *gag, pol*, and *env* regions of the genome have produced mixed results. In one study, LTR primers were able to amplify the corresponding genomic regions of 6 of 6 SRLVs comprised of 3 sheep plus 3 goat isolates of American, Dutch, and Swiss origin. In comparison, the *gag* and *pol* primers amplified 5 of 6 and 3 of 6 of these isolates respectively (22). A second study using a total of 9 isolates of North American origin (8 ovine plus 1 caprine) gave

similar outcomes for LTR (9 of 9 isolates) and *gag*-p25 (9 of 9) primers, but poor results for *gag*-p16 (1 of 9) and *env*-gp40 (2 of 9) primers (30). These results are not surprising, given lentivirus sequence variation and the fact that PCR efficiency is intimately dependent on the complementarity existing between primers and template. Quasispecies dynamics is therfore of obvious importance when designing primers for PCR-based SRLV diagnostic assays.

Serologic based tests are by far the most commonly employed for detecting SRLV-infected animals. Once again, careful consideration must be given to the nature of diagnostic reagents used, in addition to the specific test employed. As an example, a serologic assay that is widely used for detecting caprine and ovine antibodies to SRLVs is a commercially available agar gel immunodiffusion test. This test kit uses ovine progressive pneumonia virus (OPPV) as antigen. A recent study has shown however that this test is of limited value for CAE diagnosis in goats due to its low diagnostic sensitivity relative to a CAEV-based AGID test (32). The most likely reason for this low relative sensitivity was attributed to the divergence between the *gag* and *env* proteins of CAEV and OPPV. This appears not to be the case for enzyme-linked immunosorbent assays (ELISA). Highly purified recombinant gag and env proteins derived from MVV have been independently produced and evaluated for use in ELISAs in several laboratories (33–35). The performance of these proteins relative to whole-virus-based ELISAs indicates that they can be successfully used for routine serologic diagnosis of SRLV infections in sheep and goats, despite existing genomic and antigenic variablity (17,36,37). The reason that cross-reactivity is preserved against recombinant MVV antigens is not known for certain, but may be related to the inherent differences between primary and secondary binding assays or to the fact that the antigens used in these studies were purified under denaturing and reducing conditions, thus selecting for antibodies directed against highly conserved linear epitopes.

In conclusion, evidence is accumulating that the lentiviruses which infect small ruminants should be viewed as a heterogenous group or quasispecies rather than as two or more well-defined species. In addition, indirect phylogenetic and direct experimental data support the plausibility for previous, and perhaps continuing, cross-species transmission. Lastly, the foregoing arguments have obvious practical implications in the design of tests and control programs used for identifying and eradicating infected animals from flocks and herds.

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