

Maedi-visna virus infection in sheep: a review

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Titre en abrégé: Maedi-visna virus infection in sheep

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Résumé - Le virus du maedi-visna appartient au genre lentivirus de la famille des rétrovirus. Le génome de ce virus comprend trois gènes de structure codant pour l'enveloppe virale (*env*), la capsidie (*gag*) et les enzymes telles que la transcriptase réverse, l'intégrase et la dUTPase (*pol*), et plusieurs gènes accessoires: *vif*, *rev* et *tat*. Ces gènes accessoires interviennent dans la régulation de la réplication et de l'expression du virus, la modulation de son pouvoir pathogène et de son tropisme. Les organes cibles du virus maedi-visna sont par ordre d'importance le poumon, la mamelle, les articulations et le cerveau. Dans ces organes, le virus infecte les macrophages matures et entraîne des lésions de type inflammatoire contenant des lymphocytes B et T, évoluant lentement, à l'origine des signes cliniques de la maladie: essoufflement, amaigrissement, mammite, arthrites, etc... L'infection par le virus maedi-visna conduit à une réponse humorale à l'origine des tests diagnostiques actuellement disponibles: immunodiffusion en gélose, ELISA,... Ces tests sérologiques sont à la base de la plupart des plans de prophylaxie sanitaire mis en place dans de nombreux pays, en l'absence de traitement et de vaccination. Cependant de nombreuses difficultés liées à la sensibilité et à la spécificité des tests sérologiques ont conduit à s'orienter vers la mise en évidence du virus dans le sang, le lait ou les organes par des techniques d'amplification génique. Dans un futur proche, les besoins en matière de recherche sont évidents tant pour le développement de nouveaux outils diagnostiques fiables que pour une meilleure connaissance de la pathogénie du maedi-visna afin de trouver des moyens de lutte mieux adaptés.

lentivirus/ maedi-visna/ mouton/ infection lente/ revue

Summary - The maedi-visna virus (MVV) is classified as a lentivirus of the retroviridae family. The genome of MVV includes three genes: *gag*, which encodes for group-specific antigens; *pol*, which encodes for reverse transcriptase, integrase, RNase H, protease and dUTPase and *env*, the gene encoding for the surface glycoprotein responsible for receptor binding and entry of the virus into its host cell. In addition, analogous to other lentiviruses, the genome contains genes for regulatory proteins, i.e. *vif*, *rev* and *tat*. The coding regions of the genome are flanked by long terminal repeats (LTR) which play a crucial role in the replication of the viral genome and provide binding sites for cellular transcription factors. The organs targeted by MVV are, in descending order of importance, the lungs, mammary glands, joints and the brain. In these organs, the virus replicates in mature macrophages and induces slowly progressing inflammatory lesions containing B and T lymphocytes. The clinical signs of MVV infection, i.e. dyspnea, loss of weight, mastitis and arthritis, are related to the location of these lesions. Infection with MVV induces the formation of antibodies which can be detected by agar gel immunodiffusion, ELISA and the serum neutralization assay. As neither antiviral treatment nor vaccination is available, diagnostic tests are the backbone of most of the schemes implemented to prevent the spread of MVV. However, since current serological assays are still lacking in sensitivity and specificity, molecular biological methods are being developed permitting the detection of virus in peripheral blood, milk and tissue samples. Future research will have to focus on both the development of new diagnostic tests and a better understanding of the pathogenesis of MVV infection.

lentivirus/ maedi-visna/ sheep/ slow infection/ review

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1. INTRODUCTION

The lentivirus genus of the retroviridae family comprises pathogens of humans, monkeys, horses, cattle, sheep, goats and cats. The infections caused by Maedi-visna virus (MVV) in sheep and by caprine arthritis-encephalitis virus (CAEV) in goats share a number of features with the infection caused by the human immunodeficiency virus (HIV), such as an incubation period of several months or even years and a slow development of disease symptoms (Table I). The major manifestations of the diseases induced by small ruminant lentiviruses include primary interstitial pneumonia, encephalitis, lymphadenopathy, arthritis, mastitis and chronic weight loss (161, 179). Lentiviral diseases are the cause of significant economic losses incurred by the sheep and goat industries and also increasingly threaten exports of live animals.

Infections with MVV and CAEV persist for life in sheep and goats, respectively, despite a humoral and cellular immune response. The infection is characterized by progressive inflammatory lesions in various organs (3, 28, 29, 85, 143). The major, if not the sole, host cells of the virus are cells of the monocyte/macrophage cell lineage (71). In contrast to human (HIV) and simian (SIV) immunodeficiency viruses, the small ruminant lentiviruses do not infect CD4+ T lymphocytes. Therefore, the diseases they cause provide a valuable model for studying both the effects of lentivirus infection on macrophage biology and the role played by infected macrophages in the absence of immunodeficiency.

This review aims to summarize the current knowledge of the biology of MVV and its interaction with its host, the sheep. We also provide a short overview of the history of the discovery of MVV. The structure and organization of the MVV genome and of the encoded polypeptides are described, with particular emphasis on auxiliary genes. The clinical consequences of infection, the epidemiology and diagnostic tests are considered and the mechanisms of pathogenesis discussed.

2. HISTORY

MVV was discovered in sheep by Sigurdsson et al (188, 189) in Iceland in the early 50's, although the disease symptoms had been described prior to this discovery in South Africa, the USA and Iceland. The concept of « slow viruses » resulting from this discovery prompted the name of the lentivirus (*lentus* (lat.)=slow) genus of which MVV is a member. Two distinct pathological situations, corresponding to the main clinical manifestations of MVV infection, featured in those early descriptions. The first, called maedi (« dyspnea » in Icelandic), is a progressive pneumonia and the second, called visna (« fading away – state of progressive apathy » in Icelandic), is a demyelinating leukoencephalomyelitis (151). MVV can also infect other organs or tissues, particularly joints in which it causes arthritis and the mammary glands where it causes mastitis (155, 216). The properties of sheep retroviruses are summarized in Table II.

In Iceland, MVV is likely to have appeared subsequent to the importation of asymptomatic but infected Karakul rams from Germany in 1933, which resulted in widespread dissemination of the infection to most flocks. Following its discovery in Iceland, MVV infections were detected in various countries, although with differing prevalences (30, 59, 136, 166, 186, 221). Exceptions are Australia and New Zealand, where lentiviral infections have been observed in goats but not in sheep (78). In France, Russo et al (175) isolated the first French MVV strain in 1980. In North America, the North American equivalent to MVV is called ovine progressive pneumonia virus (OPPV) (48).

Complete nucleotide sequences of several MVV strains have been published since 1985, i.e., those of strains K1514 (194) from Iceland, SA-OMVV (167) from South Africa, EV1 from the UK (184) as well as clones KV1772-kv72/67 [selected for neurovirulence (5)] and LV1-1KS1 (196). More recently, a Portuguese isolate named P1OLV has been fully sequenced (10).

3. THE VIRUS

3.1 Organization of the viral genome

MV virus has a genetic organization that is typical of lentiviruses: its genome is a dimer of RNA of positive strand polarity, 9.2 Kb in size, which is reverse transcribed into proviral DNA, some of which will be integrated into the chromosomal DNA (the provirus). It comprises three structural genes, i.e. *gag* (group-specific antigen), *pol* (polymerase) and *env* (envelope), as well as various auxiliary genes. The numbers and role of the auxiliary genes vary depending on the lentivirus involved and, to a lesser degree, between the different strains of MVV.

Analogous to other retroviruses, the MVV proviral DNA is flanked by long terminal repeats (LTR) that provide the *cis* signals required for transcription, integration and polyadenylation of viral RNA (Figure 1). The LTRs play also a role in tissue tropisms (2, 4, 185) and in the replication of MVV (9).

3.2 Viral structure

MVV virions are spherical and have a unique three-layered structure. The size of the virions is about 100 nm. The central part of the virus is the genome-nucleoprotein complex, associated with the reverse transcriptase. This structure is enclosed within an icosahedral capsid surrounded by an envelope derived from the plasma membrane of the host cell.

3.2.1 Structural genes and their products (Table III)

3.2.1.1 The *gag* gene

The *gag* gene encodes for three glycoproteins from the precursor Pr55^{gag}: the capsid (p25), the nucleocapsid (p14), and the matrix (MA) protein (p17) which ensures the link between the capsid and the envelope. The MA protein is responsible for the association of the *gag* precursor with the cell plasma membrane; within the virus particle, the MA protein is localized between the viral membrane and the capsid protein of the virus. The capsid protein, the most abundant protein of the virion, forms the hydrophobic core of the virion and elicits a strong antibody response during infection which is valuable for

diagnostic tests. Within the capsid protein the nucleocapsid protein coats the viral RNA genome (103).

3.2.1.2 *The env gene*

The *env* gene encodes the glycoprotein of the virus. As for other retroviruses, the glycoprotein is synthesized as a precursor (gp160) and is cleaved by a host cell protease into two subunits: the surface glycoprotein (SU; gp135) and the transmembrane glycoprotein (TM; gp44). The TM part of the viral envelope surface glycoprotein is anchored in the lipid bilayer. The SU glycoprotein is noncovalently linked to TM. The envelope glycoproteins of lentiviruses have many important biological functions and contains the epitopes responsible for both the induction of neutralizing antibodies and the interaction of the virus with the receptor of the host cell.

Efforts to identify the receptor using overlay protein blotting assays led to MHC-II being proposed as a possible receptor (54). However, while this protein could be involved in some situations, the known distribution of the receptor is inconsistent with this hypothesis (135). Other candidates have been proposed (7, 27, 46), but, to date, the exact identity of the MVV receptor(s) is not known (93, 135); this contrasts to HIV where the receptor (CD4) and the co-receptors (receptors for chemokines) have been determined (217).

3.2.1.3 *The pol gene*

3.2.1.3.1 The reverse transcriptase

The key enzyme of retroviruses is the reverse transcriptase, a RNA-dependent DNA polymerase, encoded by the *pol* gene which permits the transcription of the viral RNA into DNA; this protein is an heterodimer which displays RNase H activity.

3.2.1.3.2 Other enzymes

3.2.1.3.2.1 *dUTPase*

In lentivirus genomes, the gene encoding the dUTPase enzyme is located in the *pol* reading frame between the RNase H and integrase coding regions. Primate lentiviruses do not possess dUTPase. This enzyme activity has been identified only in FIV (Feline Immunodeficiency Virus), EIAV (Equine

Infectious Anemia Virus), CAEV and MVV (65); dUTPase appears to decrease the frequency of G-to-A mutations. For the CAEV, *in vitro*, dUTPase deficient viruses replicate more slowly in macrophages (205); *in vivo* they are slightly attenuated (204), albeit to a lesser degree than the dUTPase-deficient EIAV (132). In contrast, dUTPase-deficient MVV appeared to be as pathogenic *in vivo* as the wild type virus (160).

3.2.1.3.2.2 *Integrase*

In each virion, numerous molecules of reverse transcriptase and integrase are associated with the viral RNA. Following reverse transcription, provirus migrates towards the nucleus, and the double-stranded DNA genome is integrated in the host cell DNA by a mechanism mediated by integrase (197). Integration involves repeated inverted sequences present in the LTR (137).

3.2.1.3.2.3 *Protease*

The viral protease cleaves the gag and gag-pol polyprotein precursors and resembles cellular aspartic acid proteases in its three-dimensional structure (103).

3.2.2 **Auxiliary genes**

The MVV has three major auxiliary genes: *tat*, *vif* (viral infectivity factor; previously called Q gene), and *rev* (regulator of virion protein expression) (Figure 1).

3.2.2.1 *Rev*

The product of the *rev* gene is a protein of 19 kDa (167 amino acids) derived from an mRNA of 1.4 kb. The MVV *rev* gene consists of four exons: a leader segment (exon 1), an untranslated portion (exon 2), and two translated exons, 3 and 4. As *rev* is an early gene of lentiviruses (139), it plays a major role in transporting unspliced mRNA from the nucleus to the cytoplasm. The *rev* protein contains nuclear export signals which permit the protein to pass through the nuclear membrane and exerts its regulatory role via a responsive element (RRE for rev responsive element) located in the *env* gene (Figure 1). The RRE is capable of binding RNA via an RNA binding site.

The essential role of *rev* is illustrated by site directed mutagenesis in the 4th exon (201): *rev*-mutagenized virus was shown to be non-infectious.

3.2.2.2 *Tat*

The *tat* gene (1.7 kb mRNA) encodes for a protein of 10 kDa which was first described for its role in stimulating gene expression directed by the viral promoter located in the 5' LTR. The Tat protein mediates the accumulation of viral mRNA via the AP-1 (Activator Protein-1) and AP-4 binding sites in the U3 region of the LTRs (70, 182) or via cellular factors such as c-Fos and c-Jun (148). A leucine-rich domain present in Tat is likely to be responsible for targeting the Tat protein at AP-1 sites in the viral LTR (34). Recently, it has been recognized that the Tat of MVV belongs to a group of Tat proteins characterized by a weak transactivation potential (210), in contrast to the Tat proteins of HIV-1 and -2, SIV or BIV, which strongly transactivate their LTRs by binding to a TAR (Tat-Activated Region) sequence. The Tat protein of MVV itself may contribute to viral pathogenesis by inducing follicular lymphoproliferative disorders in various organs (90, 209). The action of the *tat* gene may be mediated by stimulation of cellular genes, such as cytokine genes (162). A paper examining the role of *tat* by studying *tat*-deleted CAE viruses has shown that this gene is not essential for virus replication (89). It may, however, contribute to a successful interaction between the virus and its host by recruiting or modulating cellular factors involved in the initiation of transcription during the maturation process of monocytes to macrophages, which leads to increased viral gene expression *in vivo* (33). Moreover, the presence or absence of AP-1 or AP-1-like sequences may at least partially explain the differences in tropism between various MVV isolates such as EV1 (a British isolate) and SA-OMVV (a South African isolate) versus K1514 (an Icelandic isolate) (4, 198).

3.2.2.3 *Vif*

The *vif* gene encodes for a 29 kDa protein (230 aa) which, in naturally infected animals, induces a weak immune response that can be detected in Western blots (6). This protein is not homologous to cysteine protease in its entirety but contains a motif which is homologous to cysteine protease and is translated during the late stages of viral replication (6, 86). The importance of *vif* for the replication of MVV is unknown but investigations using CAEV and HIV indicate that *vif* plays a crucial role in the late stages of the viral life cycle, i.e.,

during the morphogenesis of the viral nucleoprotein core (86, 87, 92, 193). A more recent study suggests that the *vif* gene of MVV is essential for infectivity and that the Vif protein protects the viral genome from enpackaged mutagenic activities (116).

3.3 Variability and phylogeny

Genetically, lentiviruses are quite heterogeneous. This manifests itself in their antigenic diversity, differences in virulence and growth characteristics *in vitro*. This genetic plasticity is believed to contribute to viral persistence in the host animal by permitting evasion of the immune response. Moreover, antigenic diversity may also present a problem in the diagnosis of lentiviral infections and remains a formidable obstacle to vaccine development. Antigenically distinct viruses have been isolated from sheep persistently infected with MVV; these variants arise by point mutations in the *env* gene (22).

Genetic variation in MVV has been determined by PCR amplification of portions of the viral genome (35, 171, 185, 225) or by analyzing PCR products in denaturing gradient gel electrophoresis (220). These studies have allowed the extent of variability in different regions of the genome (LTR, *gag*, *pol*, *env*) to be compared and the heterogeneity of MVV strains to be determined. Moreover, these techniques permitted the phylogeny of lentiviruses to be established (Figure 2). Analysis of a 475 nt fragment in the *pol* gene of ovine lentiviruses from France revealed that the French isolates form a group closely related to the Cork CAEV strain and only distantly related to a group of ovine lentiviruses consisting of the K1514, EV1, SA-OMVV and P1OLV strains (10, 131). This analysis was confirmed by studying the sequence variability in a fragment of the *env* gene (129). Similarly, in North America, sequence analysis in the *env* gene of ovine field isolates showed them to be more similar to CAEV than to the ovine prototype strains, which suggests that ovine and caprine lentiviruses may have descended from a common caprine ancestral genotype (37). Another studies suggests that an ancestral ovine lentivirus had led to a lentivirus adapted to caprine host (206)(170). Sequence analysis of OPPV field isolates and reference isolates such as CU1, WLC1 or S93 indicate that they are slightly distinct from CAEVs and MVVs (91). In addition, this study and another suggest that OPPV may have evolved from

CAEV more than from MVV (108). This is a possibility given that lambs can be infected with CAEV by feeding them milk from infected goats. Taken together, the phylogenetic studies confirm the previously reported subdivision of the different SRLV strains into six clades (223): the caprine and ovine lentivirus sequences are interspersed in phylogenetic trees, supporting the existence of cross-species transmission (83, 142, 187).

4. VIRUS TRANSMISSION

The udder and lung are probably the two main sources of excreted virus, although other routes of infection must be also considered (24). The routes of transmission of SRLVs have been investigated and a large body of evidence has accumulated over many years; the main routes are through ingestion of infected colostrum and/or milk, or through inhalation of respiratory secretions (16, 159).

4.1 Horizontal transmission

Free virus or virus-infected cells are horizontally transmitted by inhalation of respiratory secretions (216, 230). Co-infections with other viruses or bacteria may also contribute to the spread of MVV via pulmonary exudates (151). Several studies support the hypothesis that, under certain circumstances, viral transmission between adult animals may play an important role in the spread of MVV (30). In addition, horizontal transmission is closely associated with close confinement in winter stabling (59), the duration of the presence of the virus in the flock, and annual acquisition of replacements (94, 101). Fecal contamination of drinking water has been demonstrated as a mode of MVV transmission, but to our knowledge, there are no published reports that investigate the role of other excretory products, such as saliva and urine, in the transmission of this agent (24).

4.2 Vertical transmission

Vertical transmission (including hereditary and congenital infection, and infection at parturition) is a key feature of the epidemiology of Maedi Visna (179). In an endemically infected flock, virus-infected cells and free virus are passed from ewes to their lambs via colostrum and milk (58, 59, 164, 190).

The permeability of the gut of newborn lambs greatly favours vertical transmission (94). The duration of infection in the ewe and the extent of the contamination of the progeny appear to be correlated (101). Naturally, lambing is a time of high lentivirus expression which facilitates the spread of infection (82). Since, in affected animals, mastitis is frequent, vertical transmission may be facilitated by the recruitment of mononuclear infected cells to the mammary glands (230).

4.2.1 *In utero* transmission

In utero virus transmission is a highly controversial issue (74). Despite strict lambing controls, some unexplained cases of seropositive lambs have been observed in flocks in which MVV eradication programs are carried out (96). Some authors have reported their failure to detect virus in experimentally infected Texel sheep embryos (216). Others have reported the isolation of ovine lentivirus from a 100 day-old cesarean-sectioned fetus from a naturally infected ewe (59). In an endemically infected flock, the PCR yielded positive results in peripheral blood mononuclear cells of about 10% of lambs tested prior to colostrum ingestion (23). Additional observations suggest that co-infections, such as Border disease might permit an *in utero* infection (Russo, personal communication). Embryo transfer according to International Embryo Transfer Society (IETS) protocols might be a safe way of ensuring virus-free flocks (16). For CAEV, a recent paper demonstrated the presence of CAEV-infected cells in the goat genital tract, suggesting potential for vertical transmission of CAEV from doe to embryo or fetus (68). However, for MVV, *in utero* infection may be a rare occurrence not supported by epidemiological evidence.

4.2.2 Venereal transmission

As MVV and CAEV are present in all body fluids, it might, theoretically, also be transmitted by mating (149, 203). In a preliminary study, it was impossible to infect wethers by inoculating them intraperitoneally with semen from infected rams and to date, no well-documented case of venereal transmission of MVV has been reported (de la Concha-Bermejillo, 1997)(24). Co-factors, such as infection with *Brucella ovis* or leucocytospermia of unknown origin,

may increase the shedding of virus in the semen (de la Concha-Bermejillo et al, 1996)(165).

It should be noted that due to the development of new and highly sensitive diagnostic procedures for virus detection, many older studies may have underestimated the frequency of certain modes of MVV transmission; this may be particularly relevant to *in utero* and venereal routes of transmission where the number of infected cells or quantity of cell-free virus, if present, is probably low (24).

5. PATHOGENESIS

Maedi Visna is characterized by a long incubation period and, typically, symptoms take several months or even years to develop (for a review, see Narayan and Cork, 1985) (145). Infection persists for life and infected animals are a constant reservoir of infection which, consequently, permits the virus to persist in its host.

In contrast to infections with HIV, SIV and FIV, immunosuppression is not a feature of Maedi Visna (44). This explains why secondary infections with opportunistic agents are infrequent in affected flocks. Nevertheless, the immune response to MVV exhibits certain peculiar features which may contribute to the persistence of infection. Early studies demonstrated that sheep infected with Maedi Visna virus develop a humoral immune response that is markedly slower than that directed against viruses causing acute infections (81, 111, 191). Antiviral antibodies in serum of naturally infected animals are of the IgG₁ subtype, with no detectable IgG₂ (15). However, this appears unlikely to be related to the controversial role of virus neutralization in the persistence of virus *in vivo*. Although it was demonstrated that antibodies, in principle, were also capable of neutralizing virus in macrophages, the affinity of virus binding to macrophages exceeded that of its binding to antibody. This finding prompted the suggestion that neutralizing antibodies might be unable to prevent the virus from spreading between macrophages (111). It was also argued that neutralization determinants of certain viral strains might not be detected by antibodies and that the emergence of antigenic variants might be yet another factor contributing to the antibodies' failure to achieve immunological control (42, 144).

The proliferative responses of efferent lymph cells were shown to be depressed transiently after experimental infection (14, 15). Moreover, the decrease in the numbers of CD4(+) and the increase in the numbers of CD8(+) cells caused an inversion of the ratios of CD4(+)/CD8(+) T cell populations in bronchoalveolar lavage fluids of experimentally infected sheep (138). As suggested by the presence of circulating precursors of cytotoxic T cells of the CD8(+) phenotype, however, there also exists a vigorous cell-mediated immune response to infection (17, 18). All in all, these observations indicate that certain aspects of the immune response may lack fine tuning, even if they do not suggest that the failure to eliminate the virus may per se be due to a general failure to mount an antiviral immune response. For instance, as work with other lentiviruses suggests, the failure to efficiently neutralize infection is the result of as yet undetermined mechanisms (12, 152). Particularly in the light of observations made with attenuated HIV vaccines, it seems well worth investigating whether certain immunodominant regions of Env may actually serve as decoys, thus decreasing a possible protective antibody response (69). Moreover, work with the closely related CAE virus in goats suggests that a dominant type 2 immune response may be associated with disease (158).

Other important factors contributing to viral persistence in infected sheep are the host-cell tropism and certain aspects of lentivirus-host cell interaction. Maedi Visna virus, as well as CAE virus, were shown *in vivo* to have a tropism for mononuclear phagocytes (71, 76, 121, 147). Several studies suggest that certain other cell types as dendritic cells, although less prominent, may also sustain viral replication in infected animals (181). A detailed study of the central nervous system of Icelandic sheep produced evidence of viral replication in a variety of cell types, inter alia, epithelial cells and fibroblasts of the chorioid plexus (73), and viral transcripts were detected in epithelial cells of the thyroid, kidneys and small intestines of goats infected with CAEV (232). In all lentiviruses, the restriction of virus replication depending on the developmental stage of monocyte/macrophage host cell is a key feature of virus-cell interaction. In Maedi Visna, it was demonstrated a number of years ago that viral gene expression increases during the development of the monocyte to its mature tissue form, the macrophage (72)(71). The fact that

monocytes in the blood may carry the viral genome without sustaining its replication was referred to as the 'Trojan horse mechanism', which indicates that this type of interaction with the host cell may permit the virus to be transported to tissues without being detected by the immune system (156). In this context, the observation by Brodie and coworkers (26) is of interest: in contrast to entry, viral replication in macrophages may be restricted in certain tissues. In addition, different strains of Maedi Visna Virus may, *in vivo*, differ in their host cell tropism (9, 44).

Incidentally, the tropism for cells of the monocyte lineage with an attendant lack of viral replication in monocytes also poses interesting questions regarding the evolution of the viral genome in infected animals. The evolution of the viral genome depends on several parameters, among them the error rate of the viral polymerase, evolutionary pressure exerted by functional constraints and by the immune system, and the rate and extent of viral replication. Viral variants have been shown to emerge in infected animals but there is no clear evidence that these variants arise as a result of immune pressure (134, 199)(144). Viral RNA and antigen have been demonstrated in bone marrow cells, but it has remained unclear whether the cells staining as macrophages and producing virus were in fact monocyte precursors or macrophages residing in the marrow (72).

A major difference between small ruminant lentiviruses and the immunodeficiency-causing HIV, SIV and FIV lies in the nature of the histological lesion. Even though the viruses causing immunodeficiency may induce inflammation initially, in the later stages there is extensive cell depletion (77). By contrast, both Maedi Visna and CAE viruses cause progressive inflammation. Inflammation is observed in different organs, most prominently, in the lungs and mammary glands and, less frequently, in the synovial membranes of the joints and in the brain. The extent of inflammation and the spectrum of affected organs may depend on the genetics of the infected animal as well as on the strain of the infecting virus (60). Different breeds may differ in the degree of their susceptibility to developing ovine progressive pneumonia, the North American form of Maedi Visna (50). In addition, Visna, the classical CNS form of infection originally observed in Iceland, was only rarely seen elsewhere. The mechanisms of genetically

determined resistance to clinical disease have not been determined in sheep but in goats they are linked to the MHC class I and class II antigens (174). Field isolates and established laboratory strains of Maedi Visna virus are highly heterogeneous. Undoubtedly, genetic differences are responsible for the differences in virulence between individual strains of virus although the underlying differences have not yet been determined in detail. However, work by the group of De Martini suggests that the extent of viral replication and degree of cytopathicity may be important markers of virulence, 'rapid - high' strains being more virulent than « slow-low » strains (121, 123).

The mechanisms responsible for maintaining and gradually increasing the mononuclear inflammation typical of Maedi Visna have remained an enigma. A lentivirus-specific interferon (IFN) may be one of the pro-inflammatory factors. It was described as the result of an interaction between infected macrophages and lymphocytes (146). The lentivirus-specific interferon was shown to restrict viral replication, slow down macrophage maturation, and have chemotactic properties for lymphocytes and mononuclear phagocytes (110)(231). The overall actions of the lentivirus-specific interferon would tend to aggravate and perpetuate inflammation, providing new host cells for the virus and, at the same time, preventing the virus from replicating at a high rate that might stimulate a more vigorous antiviral immune response. It should be noted, however, that this interpretation reflects an extrapolation of *in vitro* data to a situation *in vivo*, which is likely to be considerably more complex. In the light of more recent data obtained relating to HIV, it would be interesting to know whether part or all of the activity by this lentivirus-specific interferon may be related to the recently described chemokines that were shown to have a profound effect on the extent of replication of HIV (53).

Investigations on the role *in vivo* of classical type 1 and type 2 interferons and of other cytokines are scarce. Maedi Visna virus-infected sheep with severe lymphoid interstitial pneumonia had significantly elevated levels of spontaneous interferon production originating from pulmonary leukocytes, as compared to both infected animals with mild or no lesions of lymphoid interstitial pneumonia and non-infected controls. It was thought that increased local production of IFN in lentivirus-infected host tissues may serve to increase the numbers of leukocytes entering the sites of viral replication. This

promotes cell-mediated tissue damage and also provides larger numbers of cells for virus replication (122). In contrast, early recombinant ovine interferon- τ treatment decreases ovine lentivirus replication *in vivo* and prevents development of lentivirus lymphoid interstitial pneumonia (106). Infection of alveolar macrophages resulted in increased expression of interleukin-8 (IL-8) mRNA. Interestingly, mRNA of this cytokine was also demonstrated to be increased in the lungs of sheep infected with Maedi Visna virus. Expression of IL-8 correlated with the severity of the lesions. This led to the suggestion that IL-8 may play an active role in shaping the histological changes observed in the lungs of sheep suffering from Maedi Visna (127). This situation appears to differ from that found in the synovial membranes of CAE virus infected goats. Even though CAE virus caused an increased expression of IL-8 in cultured macrophages, no such effect was found in the synovial membranes (126). Since IL-8 expression was found to be increased only in those macrophages in which virus replicated at a very high rate, this difference between the status in the lungs of MV infected sheep and that in the synovial membranes of CAEV infected goats may be accounted for by a more restricted replication of CAEV in the synovial membranes of goats in contrast to a higher rate of replication of MVV in the lungs of sheep. An active role of infected macrophages in promoting inflammation is further suggested by the induction of procoagulant activity both in cultured cells and in the lungs of infected sheep (128). Recently, Woodall *et al* reported elevated levels of mRNA for granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-2 receptor (IL-2R), and interleukins 1 β , 4 and 10 (IL-1 β , IL-4, IL-10) (219). Interestingly, expression of TNF- α was not increased, which suggests that, in Maedi, not all proinflammatory cytokines are upregulated. The severity of inflammation was correlated with the viral load, indicating that the virus may be an important trigger for the formation of the lesion. In line with this interpretation, UV-inactivated virus was found to stimulate the expression of GM-CSF in *ex vivo* alveolar macrophages (219), indicating that virus replication was not required for the virus to have this effect. Infection with Maedi Visna was also shown to alter the capacity of macrophages for generating reactive oxygen species (45), which indicates that the virus alters

an important effector function implicated in the defence against microorganisms as well as in host pathology. The generation of enhanced levels in response to exogenous stimulation is of particular interest because it is indicative of 'priming', i.e. the cells show a difference in function only when responding to exogenous stimulation. This particular type of functional alteration may also occur when the cells respond to stimulation by 'physiological' signals originating from other cells *in situ*. We have recently observed that macrophages infected with CAE virus *in vitro* show altered expression of some, but not all, cytokines investigated (124)(219). Hence, such priming for an altered response may contribute to the chronic inflammation which is a hallmark of infection by small ruminant lentiviruses.

To date, the question of how the histopathological lesions typical of Maedi Visna may be generated is unresolved. *In vitro*, MVV infection and replication lead to strong cytopathic effects characterized by syncytia formation and subsequent cell lysis. The mechanism of cell death occurring during MVV infection *in vitro* is associated with the induction of apoptosis (64). *In vivo*, clearly, the mononuclear inflammation itself is the major pathogenic factor promoting some of the more obvious alterations seen in the final stages of chronic Maedi. These include, among others, lung fibrosis and its well-known manifestations such as a decrease in the gas exchange in the lungs due to a decrease in the total alveolar surface and an increase in the distance between the luminal and vascular sides of the alveoli. Lung fibrosis is a complex process, to which cytokines and other immune mediators produced during the chronic inflammation contribute. In addition, reactive oxygen species generated by macrophages and other phagocytic cells may shift the fragile protease/antiprotease balance in favor of the former which, in turn, results in enzymatic tissue destruction (102). Associated with fibrosis is a decrease in the compliance of the lung tissue which manifests as the kind of strained breathing which is called 'Maedi' by the Icelanders. Similar to other forms of lung fibrosis, bacterial infections may become more frequent and more difficult to control efficiently. In turn, such infections have an adverse effect on the health status, as do other lung diseases such as adenomatosis, an infectious tumor caused by an oncovirus (57, 150).

6. DIAGNOSIS

6.1 Antibodies and nucleic acids

Due to the persistence of circulating antibodies, the diagnosis of MVV infection is most commonly based on serological tests (208). In recent years, as an alternative to serology, methods have been developed that allow the detection of the viral genome by PCR (113, 159).

6.1.1 The first generation assays : AGID , whole-virus ELISA and immunoblot

The most widely used test is agar gel immunodiffusion (AGID), first described in the late seventies (47, 218). The antigen, a concentrate of medium harvested from cell cultures infected with the WLC1 MVV strain, contains both major structural proteins, i.e. the core protein p25 and the major envelope glycoprotein gp135. As, antigenically, MVV and CAEV are closely related (75), AGID based on this antigen has for a long time been routinely used to detect small ruminant lentivirus infections in both sheep and goats. Precipitating antibodies identified by AGID are anti-p25 with a crude concentrate and large peripheral wells (macroimmunodiffusion test) (47), and anti-gp135 with a microhexagonal well pattern, with alternate large and small peripheral wells (microimmunodiffusion test) (56, 218). Several studies demonstrated that an AGID assay with CAEV gp135 was more sensitive than an AGID assay with CAEV p25 (1, 79). Radioimmunoprecipitation assays revealed that goats infected with CAEV have much higher antibody titers against gp135 than against p25, emphasizing the necessity of using the appropriate antigen in AGID to attain a high sensitivity and specificity in the detection of antibody to CAEV or MVV (115). Hence, precipitation in an agar gel requires multiple epitope-antibody interactions, whereas the radioimmuno-precipitation assay requires only the binding to a single epitope (115). Thus, the origin and characteristics of the viral strain producing the precipitating antigen appear to be of major importance (112). However, the interpretation of the microimmunodiffusion test with gp135 antigen sometimes appears difficult or subjective and may lead to false-positive or false-negative results, although, theoretically, this assay is more sensitive than the anti-p25 AGID. In such

unclear cases, the results must be confirmed in ELISA or immunoblot (Western blot) (195).

The development of indirect ELISAs has made the diagnosis of MVV and CAEV more sensitive. The antibody response is detected at an earlier timepoint and yields a semi-quantitative assessment of the level of circulating antibodies (95, 180, 212). These first-generation ELISAs used partially or highly purified preparations of whole-virus antigens, and polyclonal enzyme conjugates, thus generating false-positive results, and necessitating a high dilution of the sera to avoid non-specific reactions.

Immunoblot techniques (Western blot) have been used to analyze the antibody responses to each of the major viral proteins, i.e. the gp135 – also referred to as gp105 (25, 107) –, the transmembrane (TM) protein gp44 (or gp55) and the internal proteins p25, p17, and p14, in sera of experimentally or naturally infected sheep (98, 104, 202) or goats (8, 213). In some cases, Western blot antibodies to p25 were detected before anti-gp135 in experimentally infected sheep, and detectable antibody responses to p14, p17 and even p25 may be absent in sheep with MVV lesions (98). Technical problems may well explain the discrepancies shown by the different reports concerning a delayed anti-gp135 response (19, 98, 107, 202). They also explain the greater sensitivity of the radioimmunoprecipitation assay in the early detection of antibodies to viral glycoproteins in naturally and experimentally infected animals (37, 75, 115, 139). Nevertheless, as the radioimmunoprecipitation assay is expensive and time-consuming, it is rarely used in routine diagnosis, but rather represents a « last resort » serological test for cases that cannot be resolved in Western blot.

The specificity of ELISA or Western blot can be improved by replacing the second antibody by protein G- or monoclonal antibodies- conjugates (19, 222, 228). Generally, a sample is considered positive in the Western blot if antibodies to at least two gene products are detected (25, 104). Nevertheless, the Western blot, although valued as a confirmation test, is not suitable for large numbers of sera, and can still yield false-positive results.

6.1.2 The second generation assays : assays based on monoclonal antibodies, recombinant proteins and peptides

By using monoclonal antibodies anti-p25 and a double-sandwich method, improved assays with a higher specificity and reliability were developed (67, 100, 168). Numerous recombinant proteins have been developed since 1990, following the initial localization of immunodominant regions in the Env and Gag proteins (117, 119). Subunits gp70 (a degradation product of either the precursor, gp160 or of SU, gp135) and gp40 (equivalent to TM glycoprotein, gp44) were expressed in *Escherichia coli*, and the entire gp70 was expressed in insect cells by a recombinant baculovirus (118). Fragments of the gp135 were expressed as fusion proteins and used to analyze the antibody response to gp135 in MVV infected sheep (32). Different segments of Gag and of the 44 kDa TM glycoprotein were expressed as glutathione S-transferase (GST) fusion proteins (163).

These recombinant proteins serve as antigens in new and promising assays (20, 61, 109, 117, 153, 163, 168, 172, 183), which are generally more sensitive and specific than whole virus tests. However, even if the results obtained with recombinant proteins turn out to be more sensitive, some non-specific reactions remain, most notably due to antibodies binding to the GST fusion partner (20). In addition, insufficiently purified antigens may lead to false positive results, making it necessary to ascertain the specificity by using double-well ELISA kits which in turn increases the cost of serology.

Synthetic oligopeptide assays, also called third-generation assays (120), have been developed, with immunodominant epitopes of the TM gp44, or using a recombinant p25 combined with a peptide from the envelope protein. In view of the cost of peptide technology, the future use of these tests in MVV eradication programs will depend on their sensitivity and specificity.

6.1.3 New developments : detection of nucleic acids

Seroconversion may take a long time, and some SRLV-infected animals may indeed fail to develop a detectable antibody response (105, 169). Moreover, as serologically positive animals may transiently become negative, novel diagnostic methods are called for to detect the presence of viral components in cells or tissues.

In recent years, the PCR, known for its high sensitivity, has been applied to the diagnosis of lentivirus infection in sheep and goats to detect DNA and RNA in peripheral blood and tissues. Preliminary reports have demonstrated proviral DNA in cultured cells within 24 h post infection (226). These reports have also stressed the importance of selecting primers that take into account that different regions of the viral genome differ in the degree of heterogeneity. In most studies, conserved regions of LTR, and of *gag* or *pol* genes were targeted for PCR (226, 227). PCR can be used for the direct detection of MVV in clinical specimens, either prior to culture or after cocultivation with susceptible cells. The latter is more sensitive as only a few PBMCs are infected by small ruminant lentiviruses *in vivo*. In experimentally infected animals, PCR seems to be more sensitive than serology, presumably because primers can be used that are perfectly complementary to the nucleotide sequences of the virus selected for infecting the animals (Vitu *et al*, submitted). Conversely, results reported of cases of natural small ruminant lentivirus infections indicated that direct PCR on PBMC, when compared with serological tests, might fail in a variable proportion of seropositive animals (169, 215, 224). Moreover, direct PCR yields a positive result only in animals exhibiting a high virus load (25).

Attempts at PCR diagnosis on milk or derived products, based on DNA and RNA extraction, showed mixed results (8, 66, 130, 169, 177, 224). In addition to the presence of inhibitory contaminants, an intermittent shedding of virus-infected cells in milk is suggested, which would limit the application of PCR in field conditions (211).

PCR diagnosis has been improved by a selection of degenerate primer sets and by nested, semi-nested and double-nested procedures (130, 131, 215) and/or by using quantitative PCR analysis (80, 229). Southern-blot hybridization with suitable probes is more sensitive than ethidium bromide staining (169, 176), and significantly increases the sensitivity and specificity, especially when samples are analyzed without prior *in vitro* culture of cells. Results obtained with PCR techniques indicated that seroconversion can be delayed for several months following natural infection with CAEV (169). However, the PCR test remains expensive and labor-intensive, and its performance will have to be evaluated in a greater number of field samples

before it can become the « gold standard » for detecting MVV infection, which, theoretically, should be possible because it can demonstrate the presence of a low number of target sequences.

The *in situ* PCR has been adapted to amplify viral DNA in fixed cells, thus facilitating identification of latent infection of cells (84, 165). *In situ* hybridization, which detects MVV RNA in cultured cells, has been reported to be as sensitive as PCR, and cocultivation studies in latently infected seronegative sheep suggest that the infection frequently remains undetected by serological tests (104). Combined with immunocytochemistry, *in situ* hybridization permits the simultaneous detection of MVV antigens and RNA within the same cell (173). *In situ* hybridization is a complementary technique of classical histopathology, but is more useful in experimental studies than as a diagnostic tool.

6.2 Other diagnostic methods

In cases of clinical disease, gross and microscopic post mortem pathology indicates the presence of histological alterations in target organs, i.e., interstitial pneumonia with a predominant mononuclear cellular infiltration (11, 28, 143), presence of lymphoid follicles in the udder parenchyma and adjacent to secretory ducts associated with an increased number of T lymphocytes and macrophages in milk cell counts (49, 62, 82, 133, 207), or in joints (52, 85, 125).

Virus isolation is a delicate technique: tissue samples taken from an infected animal must contain living cells for cocultivation on sheep choroid plexus cells or goat synovial membrane cells which also support the replication of MVV (104). The cocultivation can also be performed with PBMCs or milk leucocytes. Explant cultures of affected tissue are carried out after necropsy. A cytopathic effect with formation of giant multinucleated cells (syncytia) is expected after two to three weeks, but some ovine lentivirus strains may not be detected due to their failure to induce a clear cytopathic effect or because the virus remains latent (38). In doubtful cases, cell staining, immunocytochemistry, electron microscopy, or reverse-transcriptase tests are performed (139).

7. PREVENTION

7.1 Present methods

In most countries, both MVV and CAEV infections in sheep and goats are currently controlled by an array of complementary methods (63, 96, 97, 99, 192). Periodic serological tests using agar gel immunodiffusion (AGID) or ELISA represent the standard method of detecting MVV-infected animals. For the eradication of infection in the flocks, two different strategies are adopted. Because, as described above, colostrum and milk are of prime importance in the infection of newborn lambs or kids, the lambs are removed from their infected mothers immediately after birth and are raised in separate flocks. Colostrum fed to these lambs is heat-treated (56°C for 60 min) and milk is pasteurized (96). Better still, the animals are fed colostrum and milk from certified MV-free ewes. As an alternative to separating newborn lambs from their mothers, seropositive animals may be removed from the flock.

Although these methods have met with some success in several countries, these control and eradication programs have a number of limitations, such as insufficient sensitivity and specificity of serological tests, relative importance of the other modes of transmission or resistance to culling seropositive animals particularly in flocks raised for commercial milk production (159).

The risk of resistant MVV strains emerging and the cost involved have prevented the development of chemotherapy for treating small ruminant lentivirus infections. The only use of antiviral drugs in maedi-visna infection is to provide a model for *in vivo* testing of candidate anti-HIV drugs (200).

7.2 Vaccination

Not surprisingly, vaccines for MV are not currently available due to the formidable difficulties presented by the biology of the interaction between the lentivirus and its host, e.g. genetic variation of the virus, the complex mechanisms of immunoprotection and viral persistence (141, 154). These obstacles to vaccine development highlight the importance of prevention.

Despite the prospects of developing an effective vaccine against MVV and CAEV in sheep and goats remaining poor (161), a number of research groups

continue to defend the feasibility of a search for such a vaccine (13, 39-41, 88, 154, 157), advocating different strategies. The use of intact inactivated virions does not seem commendable due to inefficacy and a debatable risk of such a product inducing even more severe symptoms and lesions (51, 140, 178). In contrast, the use of attenuated viruses obtained by deletion of selected genes, i.e., *vif*, *tat* and dUTPase, looks promising. The protective role these mutants play can be determined after either conventional or DNA vaccination. Bacterial or viral vehicles to produce recombinant vaccines have turned out to be another promising approach (21, 31, 55).

7.3 Other control methods

The selection of sheep with a natural resistance to MVV may offer another approach to controlling MVV. A few studies have reported a possible natural resistance to the disease in some breeds (50, 157). A high seroprevalence has been demonstrated in the Texel, Border Leicester and Finnish Landrace breeds (214, 230), as opposed to the Ile-de-France breed (101). It seems that there exists a genetic susceptibility to the disease rather than a susceptibility to the infection per se (60, 230). The most promising strategy would be to learn more about the immune mechanisms involved in natural resistance to the disease at the level of the breed as a whole and at that of the individual animal. Breeding transgenically resistant sheep, even if theoretically possible (43), appears, in practice, not an achievable goal.

8. CONCLUSION

- In view of the impact of MVV on the farming sector of most countries, the disease is classified in list B of diseases by the Office International des Epizooties (114). A continuation of research is therefore called for in order to increase our knowledge. Inter alia, the genetic variability of ovine and caprine lentiviruses in both natural and experimental infections must be analysed with a view to developing tools permitting more refined methods of diagnosing lentiviral infections. Farmers and veterinary authorities anxious to improve their MVV eradication programs would welcome such tools (159).

- In addition, the viral determinants of virulence and the pathogenesis of ovine lentivirus in its natural host must be elucidated, not least to improve our understanding of the disease mechanisms underlying HIV, particularly macrophage-tropic HIV. In this context, the determination of cell receptors for MVV infection as has recently been achieved in the case of HIV, SIV and FIV (217), would constitute a major scientific advance.
- Ultimately, vaccination strategies need to be developed in order to extend the armamentarium available to control maedi-visna in sheep.

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FIGURE LEGENDS

Figure 1 - Genomic organization of Maedi-visna virus

Figure 2 - Phylogenetic tree of various retroviruses (HTLV1: human T lymphotropic lymphoma virus; BLV: bovine leukaemia virus; EIAV: equine infectious anemia virus; CAEV: caprine arthritis-encephalitis virus; MVV: maedi-visna virus; FIV: feline immunodeficiency virus; SIVcpz: simian immunodeficiency virus of chimpanzees; HIV-1: human immunodeficiency virus 1; SIVagm: simian immunodeficiency virus of African green monkeys; HIV-2: human immunodeficiency virus 2; BIV: bovine immunodeficiency virus; JDV: Jembrana disease virus). Adapted from Chadwick *et al* (36)

Table I. The subfamily of lentiviruses and related viruses

VIRUS	NATURAL HOST	MAIN TARGET CELL	CLINICAL MANIFESTATIONS
<i>TRUE LENTIVIRUSES</i>			
Maedi-visna virus (MVV)	Sheep	monocyte/macrophage	pneumonia, encephalitis, mastitis, arthritis
Caprine arthritis-encephalitis virus (CAEV)	Goat	monocyte/macrophage	pneumonia, encephalitis, mastitis, arthritis
Equine infectious anaemia virus (EIAV)	Horse	monocyte/macrophage	fever, anaemia, asymptomatic carriers
Human immunodeficiency virus (HIV)	Human	lymphocyte CD4+, monocyte/macrophage	immune deficiency, encephalopathy, myelopathy, opportunist infections (AIDS)
Feline immunodeficiency virus (FIV)	Cat	CD4+ and CD8+ T lymphocytes, B lymphocyte, monocyte/macrophage	immune deficiency, opportunistic infections
Simian immunodeficiency virus (SIV)	Monkey	lymphocyte, monocyte/macrophage	immune deficiency, opportunistic infections
Bovine immunodeficiency virus (BIV)	Cattle	T lymphocyte, B lymphocyte, $\gamma\delta$ Tcell, monocyte/macrophage	silent infection, immune deficiency?
<i>RELATED LENTIVIRUSES</i>			
Jembrana disease virus (JDV)	Cattle	?	acute and severe disease: fever, lymphadenopathy, lymphopenia; pathology: intense lymphoproliferative disorder

Table III. Organization of the maedi-visna virus

Gene	Product	
	Precursor	Final products
<i>gag</i> (group-specific antigen)	Pr55 ^{gag}	matrix (MA): p17 capsid (CA): p25 nucleocapsid (NC): p14
<i>pol</i> (polymerase)	gag-pol polyprotein precursor	reverse transcriptase (RT) integrase (IN) protease (PR) Rnase H dUTPase
<i>vif</i> (viral infectivity factor)	...	Vif
<i>tat</i>	...	Tat
<i>Rev</i> (regulator of virion protein expression)	...	Rev
<i>env</i> (envelope)	Env: gp160	surface glycoprotein (SU): gp135 transmembrane glycoprotein (TM): gp44

Table II. Retroviruses of sheep

VIRUS	TYPE OF RETROVIRUS	SYNONYMS OF THE DISEASE	CLINICAL MANIFESTATIONS
Maedi-visna virus (MVV)	Lentivirus	maedi, visna, zwoegerziekte, la bouhite, ovine progressive pneumonia	pneumonia (maedi), encephalitis (visna), mastitis (hard udder), arthritis
Jaagsiekte sheep retrovirus (JSRV)	Type D/B retrovirus	sheep pulmonary adenomatosis (SPA), ovine pulmonary carcinoma (OPC)	contagious lung neoplasm: dyspnoea, moist rales, coughing and production of an abundant pulmonary fluid
Enzootic nasal tumors virus (ENTV)	Type D/B retrovirus (related to JSRV)	enzootic nasal tumor	adenopapilloma or adenocarcinoma (origin from the olfactory mucosa of the turbinate region)

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