Maedi-visna virus infection in sheep: a review

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Review article

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Michel Pépin[a]*, Christian Vitu[a], Pierre Russo[a], Jean-François Mornex[b], Ernst Peterhans[c]

[a] Unité pathologie des petits ruminants, Cneva Sophia-Antipolis, BP 111, 06902 Sophia-Antipolis cedex, France
[b] Inra-ENV Lyon, Marcy-l'Étoile, France
[c] Institute of Veterinary Virology, Bern, Switzerland

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Abstract – 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. © Inra/Elsevier, Paris

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

Résumé – L'infection par le virus Maedi-visna chez le mouton : une revue. Le virus du maedi-visna appartient au genre lentivirus de la famille des rétrovirus. Le génome de ce virus com-

* Correspondence and reprints
Tel.: (33) 4 92 94 37 00; fax: (33) 4 92 94 37 01; e-mail: vasa20@calva.net

prend trois gènes de structure codant pour l'enveloppe virale (*env*), la capside (*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, etc. 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

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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 (Russo et al., 1991; Phelps and Smith, 1993). 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

Table I. The subfamily of lentiviruses and related viruses.

Virus	Natural host	Main target cell	Clinical manifestations
True lentiviruses			
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, γδ Tcell, monocyte/macrophage	subclinical infection, immune deficiency?
Related lentiviruses			
Jembrana disease virus (JDV)	cattle	?	acute and severe disease: fever, lymphadenopathy, lymphopenia; pathology: extensive lymphoproliferative disorder (Chadwick et al., 1995)

lesions in various organs (Cadoré et al., 1996; Harkiss et al., 1991; Mornex et al., 1994; Anderson et al., 1994; Cadoré et al., 1993). The major, if not the sole, host cells of the virus are cells of the monocyte/macrophage cell lineage (Gendelman et al., 1986). 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. (1952, 1957) in Iceland in the early 1950s, 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 (Palsson, 1976; Mornex et al., 1994; Cadoré et al., 1996) and the second, called visna ('fading away – state of progressive apathy' in Icelandic), is a demyelinating leukoencephalomyelitis (Palsson, 1976; Watt et al., 1994). MVV can also infect other organs or tissues, particularly joints in which it causes arthritis (Watt et al., 1994) and the mammary glands where it causes mastitis (Pekelder, 1993; Watt et al., 1994). The properties of sheep retroviruses are summarized in *table II*.

In Iceland, MVV is likely to have appeared subsequent to the importation of infected asymptomatic 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 (Madewell et al., 1987; Campbell et al., 1994; Schaller et al., 1995; Pritchard et al., 1995; de la Concha-Bermejillo, 1997). Exceptions are Australia and New Zealand, where lentiviral infections have been observed in goats but not in sheep (Greenwood et al., 1995). In France, Russo et al. (1980) isolated the first French MVV strain in 1980.

Complete nucleotide sequences of several MVV strains have been published since 1985, i.e. those of strains K1514 (Sonigo et al., 1985) from Iceland, SA-OMVV (Quérat et al., 1990) from South Africa, EV1 from the UK (Sargan et al., 1991) as well as clones KV1772-kv72/67 [selected for neurovirulence (Andresson et al., 1993)] and LV1-1KS1 (Staskus et al., 1991).

3. THE VIRUS

3.1. Viral structure

MVV virions are spherical and have a unique three-layered structure. The size of the virions is about 100 nm. The central

Table II. Retroviruses of sheep.

Virus	Type of retrovirus	Synonyms of the disease	Clinical manifestations	Reference
Maedi-visna virus(MVV)	lentivirus	maedi, visna, zwoegerziekte, la bouhite, ovine progressive pneumonia	pneumonia (maedi), encephalitis (visna), mastitis (hard udder), arthritis	(Narayan and Cork, 1985; Clements and Zink, 1996; Russo et al., 1991)
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 abundant pulmonary fluid	(Hecht et al., 1996; Palmarini et al., 1996; York et al., 1992; Quérat et al., 1987)
Enzootic nasal tumors virus (ENTV)	type D/B retrovirus (related to JSRV)	enzootic nasal tumor	adenopapilloma or adeno-carcinoma (originating from the olfactory mucosa of the turbinate region)	(Cousens et al., 1996)

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. 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. It comprises three structural genes, i.e. *gag* (group-specific antigen), *pol* (polymerase) and *env* (envelope), as well as various auxiliary genes. 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 numbers and role of the auxiliary genes vary depending on the lentivirus involved and, to a lesser degree, between the different strains of MVV.

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

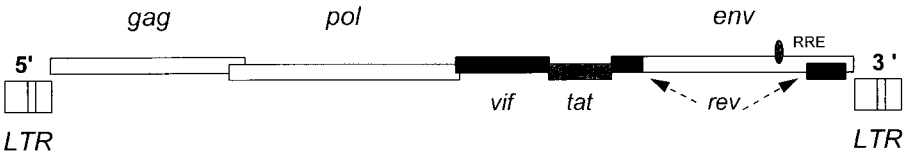

Figure 1. Genomic organization of maedi-visna virus.

Table III. Organization of the maedi-visna virus.

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

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 (Joag et al., 1996).

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 non-covalently linked to TM. The envelope glycoproteins of lentiviruses have many important biological functions and contain the epitopes

responsible for both the induction of neutralizing antibodies and the interaction of the virus with the receptor of the host cell. To date, no surface receptors for MVV have been conclusively defined although cellular proteins able to bind to the virus have been described (Dalziel et al., 1991; Clements and Payne, 1994); this contrasts to HIV where the receptor (CD4) and the co-receptors (receptors for chemokines) have been determined (Willett et al., 1997).

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 lack dUTPase. This enzyme activity has been identified only in FIV (feline immunodeficiency virus), EIAV (equine infectious anemia virus), CAEV and MVV (Elder et al., 1992); dUTPase appears to decrease the frequency of G-to-A mutations. In vitro, dUTPase deficient CAE viruses replicate more slowly in macrophages (Turelli et al., 1996). In vivo they are slightly attenuated (Turelli et al., 1997), albeit to a lesser degree than the dUTPase-deficient EIAV (Steagall et al., 1995; Lichtenstein et al., 1997). In contrast, dUTPase-deficient MVV appeared to be as pathogenic in vivo as the wild type virus (Petursson et al., 1998).

3.2.1.3.2.2. Integrase

In each virion, numerous molecules of reverse transcriptase and integrase are associated with the viral RNA (Marin et al., 1994). 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 (Stormann et al., 1995). Integration involves repeated inverted sequences present in the LTR (Marin et al., 1994).

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 (Joag et al., 1996).

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) (Clements and Zink, 1996) *(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 (Mazarin et al., 1990), 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 to exert 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

Table IV. Requirement of the *rev* gene of visna virus for productive infection (adapted from Toohey and Haase, 1994).

Parameter	Replication-competent DNA (subclone LV1-KS1)	*rev* mutant	Complementation assay[a]
Cytopathic effects in SCP[b] cells	++++	0	ND[d]
RT[c] activity	++++	0	++
Percentage of cells with visna virus RNA (by in situ hybridization)	0.39 %	1.0×10^{-4} %	ND

[a] The *rev* function was restored by providing *rev* in trans;[b] SCP = sheep chorioid plexus; [c] RT = reverse transcriptase; [d] ND = not done.

exon (Toohey and Haase, 1994): *rev*-mutagenized virus was shown to be non-infectious *(table IV)*.

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 (Gdovin and Clements, 1992; Saltarelli et al., 1993) or via cellular factors such as c-Fos and c-Jun (Neuveut et al., 1993). 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 (Carruth et al., 1996). Recently, it has been recognized that the Tat of MVV belongs to a group of Tat proteins characterized by a weak transactivation potential, 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 (Hayman et al., 1993; Vellutini et al., 1994). The action of the *tat* gene may be mediated by stimulation of cellular genes, such as cytokine genes (Philippon et al., 1994). A recent paper examining the role of *tat* by studying *tat*-deleted CAE viruses has shown that this gene is not essential for virus replication (Harmache et al., 1995b). 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 (Carruth et al., 1994). 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) (Andresdottir et al., 1994; Sutton et al., 1997).

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 (Audoly et al., 1992). 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 (Audoly et al., 1992; Harmache et al., 1995a). 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 (Hoglund et al., 1994; Harmache et al., 1996a; Simon et al., 1997).

3.3. Variability

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 (Braun et al., 1987).

Genetic variation in MVV has been determined by PCR amplification of portions of the viral genome (Zanoni et al., 1992; Sargan et al., 1995; Rosati et al., 1995) or by analysing PCR products in denaturing gradient gel electrophoresis (Woodward et al., 1994). 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-

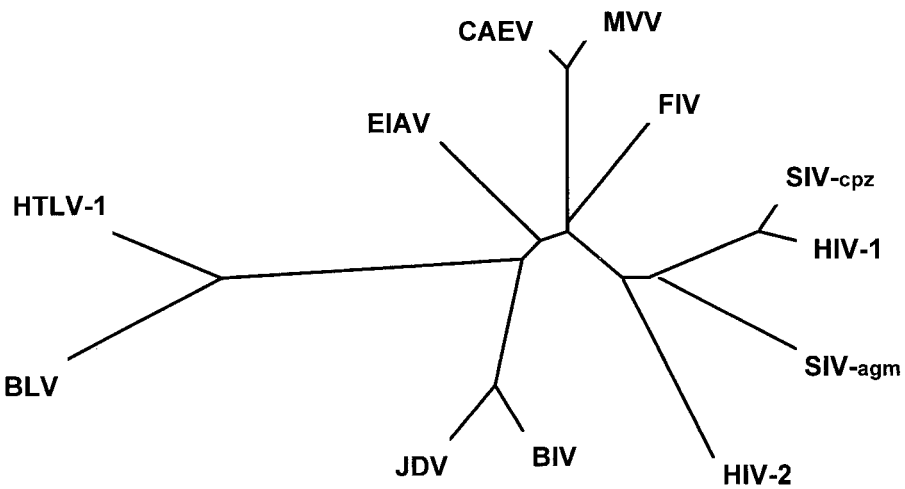

Figure 2. Phylogenetic tree of various retroviruses (HTLV1: human T lymphotropic lymphoma virus; BLV: bovine leukaemia virus; EIAV: equine infectious anemia virus; CAEV: caprine arthritis-encephalitis virus; MVV: maedi-visna virus; FIV: feline immunodeficiency virus; SIVcpz: simian immunodeficiency virus 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 (from Chadwick et al., 1995).

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 and SA-OMVV strains. This analysis was confirmed by studying the sequence variability in a fragment of the *env* gene (Leroux et al., 1995). 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 (Cheboune et al., 1996). Another study suggests that CAEV originated from an ancestral ovine lentivirus adapted to a caprine host (Valas et al., 1997).

4. VIRUS TRANSMISSION

4.1. Horizontal transmission

Free virus or virus-infected cells are horizontally transmitted by inhalation of respiratory secretions (Zink and Johnson, 1994; Watt et al., 1994). Co-infections with other viruses or bacteria may also contribute to the spread of MVV via pulmonary exudates (Palsson, 1976). Several studies support the hypothesis that, under certain circumstances, viral transmission between adult animals may play an important role in the spread of MVV (Campbell et al., 1994). In addition, horizontal transmission is closely associated with close confinement in winter stabling (de la Concha-Bermejillo, 1997), the duration of the presence of the virus in the flock (Houwers et al., 1989), and annual acquisition of replacements (Houwers, 1997).

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 (Russo et al., 1991). In an endemically infected flock, virus-infected cells and free virus are passed from ewes to their lambs via colostrum and milk (Watt et al., 1994; de la Concha-Bermejillo, 1997). The permeability of the gut of newborn lambs greatly favours vertical transmission (Houwers, 1997). The duration of infection in the ewe and the extent of the contamination of the progeny appear to be correlated (Houwers et al., 1989). Naturally, lambing is a time of high lentivirus expression which facilitates the spread of infection (Guiguen et al., 1992). Since mastitis is frequent in affected animals, vertical transmission may be facilitated by the recruitment of mononuclear infected cells to the mammary glands (Zink and Johnson, 1994).

4.2.1. In utero transmission

In utero virus transmission is a highly controversial issue. Despite strict lambing controls, some unexplained cases of seropositive lambs have been observed in flocks in which MVV eradication programs are carried out (Houwers et al., 1983). Some authors have reported their failure to detect virus in experimentally infected Texel sheep embryos (Watt et al., 1994). Others have reported the isolation of ovine lentivirus from a 100-day-old cesarean-sectioned fetus from a naturally infected ewe (de la Concha-Bermejillo, 1997). 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 (Brodie et al., 1994). Additional observations suggest that co-infections, such as Border disease, might permit an in utero infection (Russo, pers. comm.).

Embryo transfer might be a safe way of ensuring virus-free flocks (Watt et al., 1994). However, in utero infection may be a rare occurrence and is not supported by epidemiological evidence (Houwers et al., 1989).

4.3. Venereal transmission

As the virus is present in all body fluids, it might, theoretically, also be transmitted by mating. However, to date no well-documented case of venereal transmission has been reported (de la Concha-Bermejillo, 1997). 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).

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). 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 (Clements and Zink, 1996). This explains why secondary infections with opportunistic agents are infrequent in affected flocks. Nevertheless, the immune response to maedi-visna virus 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 (Gudnadottir and Kristinsdottir, 1967; Sihvonen, 1980; Kennedy-Stoskopf and Narayan, 1986). Antiviral antibodies in serum of naturally infected animals are of the IgG_1 subtype, with no detectable IgG_2 (Bird et al., 1995). 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 (Kennedy-Stoskopf and Narayan, 1986). 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 (Narayan et al., 1987; Clements et al., 1988).

Due to the tropism for mononuclear phagocytes, lymphoid tissues are considered to be important targets for the replication of MVV. Indeed, elegant studies using lymph node cannulation techniques have yielded important information with regard to virus–host interaction. The proliferative responses of efferent lymph cells were shown to be depressed transiently after experimental infection (Bird et al., 1993, 1995). 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 (Maslak and Schmerr, 1993). 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 (Blacklaws et al., 1994, 1995). 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 (Pancino et al., 1994; Bertoni et al., 1994). 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 (Garrity et al., 1997). Moreover, work with the closely related CAE virus in goats suggests that a dominant type 2 (i.e. antibody centered) immune response may be associated with disease (Perry et al., 1995).

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 (Narayan et al., 1982; Gendelman et al., 1985; Lairmore et al., 1987). Several studies suggest that certain other cell types, although less prominent, may also sustain viral replication in infected animals. 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 (Georgsson et al., 1989), and viral transcripts were detected in epithelial cells of the thyroid, kidneys and small intestines of goats infected with CAEV (Zink et al., 1990). Very recently, endothelial cells were shown to support the replication of MVV in vitro, with interesting differences depending on the origin of the tissue used to prepare the endothelial cells (Craig et al., 1997).

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 (Gendelman et al., 1985). 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 (Peluso et al., 1985). In this context, the observation by Brodie and coworkers (Brodie et al., 1995) 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 (Clements and Zink, 1996).

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 (Lutley et al., 1983; Thormar et al., 1983; Narayan et al., 1987). 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 (Gendelman et al., 1985).

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 (Gougeon et al., 1993). 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 (DeMartini et al., 1991). Different breeds may differ in the degree of their susceptibility to developing ovine progressive pneumonia, the North American form of maedi visna (Cutlip et al., 1986). 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 (Ruff et al., 1993). Field isolates and established laboratory strains of maedi-visna virus are highly heterogeneous (Quérat et al., 1984; Sargan et al., 1991; Zanoni et al., 1992; Andresdottir et al., 1994; Carey and Dalziel, 1994; Leroux et al., 1996). 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 (Lairmore et al., 1987, 1988b).

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 (Narayan et al., 1985). The lentivirus-specific interferon was shown to restrict viral replication, slow down macrophage maturation, and have chemotactic properties for lymphocytes and mononuclear phagocytes (Kennedy et al., 1985; Zink and Narayan, 1989). 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 (D'Souza and Harden, 1996).

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 (Lairmore et al., 1988a). 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 (Legastelois et al., 1996). 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 (Lechner et al., 1997b). 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 (Lena et al., 1994). Recently, Woodall et al. reported elevated levels of mRNA for granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-2 receptor (IL-2R), and interleukins 1β, 4 and 10 (IL-1β, IL-4, IL-10) (Woodall et al., 1997). Interestingly, expression of TNF-α was not increased, which suggests that, in maedi, not all pro-inflammatory 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 (Woodall et al., 1997), 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 (Cottin et al., 1996), 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 (Woodall et al., 1997; Lechner et al., 1997a). 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. 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 favour of the former which, in turn, results in enzymatic tissue destruction (Hutchison, 1987). 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 (Snyder et al., 1983, Dawson et al., 1990).

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. In recent years, as an alternative to serology, methods have been developed that allow the detection of the viral genome by PCR.

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 1970s (Cutlip et al., 1977; Winward et al., 1979). 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 (Dawson et al., 1996). As, antigenically, MVV and CAEV are closely related (Gogolewski et al., 1985), 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) (Cutlip et al., 1977), and anti-gp135 with a microhexagonal well pattern, with alternate large and small peripheral wells (microimmunodiffusion test) (Winward et al., 1979; Dawson et al., 1982). Several studies demonstrated that an AGID assay with CAEV gp135 is more sensitive than an AGID assay with CAEV p25 (Grewal, 1986; Adams and Gorham, 1986). 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 (Knowles et al., 1994). Hence, precipitation in an agar gel requires multiple epitope–antibody interactions, whereas the radioimmunoprecipitation assay requires only the binding to a single epitope (Knowles et al., 1994). Thus, the origin and characteristics of the viral strain producing the precipitating antigen appear to be of major importance (Klein et al., 1985). 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 unclear cases, the results must be confirmed in ELISA or immunoblot (western blot).

The development of indirect ELISAs has improved the diagnosis of MVV and CAEV. The antibody response is detected at an earlier timepoint and yields a semiquantitative assessment of the level of circulating antibodies (Vitu et al., 1982;

Houwers et al., 1982; Russo et al., 1988). 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 analyse the antibody responses to each of the major viral proteins, i.e. the gp135 – also referred to as gp105 (Kajikawa et al., 1990; Brodie et al., 1992), the transmembrane (TM) protein gp44 (or gp55) and the internal proteins p25, p17 and p14, in sera of experimentally or naturally infected sheep (Houwers and Nauta, 1989; Johnson et al., 1992; Torfason et al., 1992) or goats (Vitu et al., 1993; Barlough et al., 1994). In some cases, in the western blot, antibodies to p25 were detected before anti-gp135 in experimentally infected sheep (Houwers and Nauta, 1989), and detectable antibody responses to p14, p17 and even p25 may be absent in sheep with MVV lesions (Houwers and Nauta, 1989). Technical problems may well explain that different authors recorded differing results concerning a delayed anti-gp135 response (Houwers and Nauta, 1989; Bosgiraud et al., 1989; Kajikawa et al., 1990; Torfason et al., 1992). 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 (Gogolewski et al., 1985; Mazarin et al., 1990; Knowles et al., 1994; Chebloune et al., 1996). 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 conjugates of protein G or monoclonal antibody conjugates (Bosgiraud et al., 1989; Zanoni et al., 1989, 1994). Generally, a sample is considered positive in the western blot if antibodies to at least two gene products are detected (Brodie et al., 1992; Johnson et al., 1992). 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 (Houwers and Schaake, 1987; Reyburn et al., 1992). Numerous recombinant proteins have been developed since 1990, following the initial localization of immunodominant regions in the Env and Gag proteins (Kwang and Cutlip, 1992; Kwang et al., 1996). The 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 (Kwang et al., 1995). Fragments of the gp135 were expressed as fusion proteins and used to analyse the antibody response to gp135 in MVV-infected sheep (Carey et al., 1993). Different segments of Gag and of the 44 kDa TM glycoprotein were expressed as glutathione S-transferase (GST) fusion proteins (Power et al., 1995).

These recombinant proteins serve as antigens in new and promising assays (Reyburn et al., 1992; Kwang and Cutlip, 1992; Zanoni et al., 1994; Rosati et al., 1994; Power et al., 1995; Keen et al., 1995; 1996; Boshoff et al., 1997), 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 nevertheless remain, most notably due to antibodies binding to the GST fusion partner (Boshoff et al., 1997). In addition, insufficiently purified antigens may lead to false positive results, making it necessary to check the specificity by using double-well ELISA kits which in turn increases the cost of serology.

Synthetic oligopeptide assays, also called third-generation assays (Kwang and Torres, 1994), 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 programmes will depend on their sensitivity and specificity.

6.1.3. New developments: detection of nucleic acids

Seroconversion may take a long time, and some infected animals may indeed fail to develop a detectable antibody response. 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 (Zanoni et al., 1990b). 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 (Zanoni et al., 1990a, b). 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., 1997). Conversely, results reported of cases of naturally infected animals indicated that direct PCR on PBMCs, when compared with serological tests, might fail in a number of seropositive animals (Rimstad et al., 1993; Zanoni et al., 1996; Vitu et al., 1997). Moreover, direct PCR yields a positive result only in animals exhibiting a high virus load (Brodie et al., 1992).

Attempts at PCR diagnosis of milk, based on DNA and RNA extraction, showed mixed results (Rimstad et al., 1993; Barlough et al., 1994; Zanoni et al., 1996; Vitu et al., 1997; Leroux et al., 1997). 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 (Vitu et al., 1997).

PCR diagnosis has been improved by an array of degenerate primer sets and by nested, semi-nested and double-nested procedures (Leroux et al., 1995; Barlough et al., 1994; Vitu et al., 1997; Leroux et al., 1997). Southern-blot hybridization with suitable probes is more sensitive than ethidium bromide staining (Rimstad et al., 1993; Russo et al., 1997), and significantly increases the sensitivity and specificity, especially when samples are analysed 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 (Rimstad et al.,

1993). However, the PCR test remains expensive and labour-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 the identification of latent infection of cells (Haase et al., 1990). In situ hybridization, which detects MVV RNA in cultured cells, has been reported to be as sensitive as PCR, and cocultivation studies using cells of latently infected seronegative sheep suggest that the infection frequently remains undetected by serological tests (Johnson et al., 1992). Combined with immunocytochemistry, in situ hybridization permits the simultaneous detection of MVV antigens and RNA within the same cell (Roy et al., 1992). 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 (Mornex et al., 1994; Cadoré et al., 1996), presence of lymphoid follicles in the udder parenchyma and adjacent to secretory ducts (Lujan et al., 1991) associated with an increased number of T lymphocytes and macrophages in milk cell counts (Guiguen et al., 1992).

Virus isolation is a delicate technique: tissue samples taken from an infected animal must contain living cells for cocultivation on sheep chorioid plexus cells or goat synovial membrane cells which also support the replication of MVV (Johnson et al., 1992). 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 2–3 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 (Chebloune et al., 1996). In doubtful cases, cell staining, immunocytochemistry, electron microscopy, or reverse-transcriptase tests are performed (Mazarin et al., 1990).

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 (Dion, 1991). 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 at birth and raised in separate flocks. Colostrum fed to these lambs is heat-treated (56 °C for 60 min) and milk is pasteurized (Houwers et al., 1983). Preferably, however, 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 programmes 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.

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 (Thormar et al., 1995).

7.2. Vaccination

Not surprisingly, vaccines for MVV 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 persistance (Pearson et al., 1989; Montelaro et al., 1989). 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 remain poor (Phelps and Smith, 1993), a number of research groups continue to defend the feasability of a search for such a vaccine (Pearson et al., 1989; Cheevers et al., 1994; Perk et al., 1996; Harmache et al., 1996b), 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 (McGuire et al., 1986; Russo et al., 1993). 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 (Perk et al., 1996; Harmache et al., 1996b). Bacterial or viral vectors to produce recombinant vaccines have turned out to be another promising approach (Cardenas and Clements, 1992; Bourgogne et al., 1996; Xu et al., 1997).

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 (Perk et al., 1996). A high seroprevalence has been demonstrated in the Texel, Border Leicester and Finnish Landrace breeds (Vorster et al., 1996; Zink and Johnson, 1994), as opposed to the Ile-de-France breed (Houwers et al., 1989). It seems that there exists a genetic susceptibility to the disease rather than a susceptibility to the infection per se (Zink and Johnson, 1994). 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 (Clements et al., 1994), appears, in practice, not to be 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 (Dawson et al., 1996). 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 programmes would welcome such tools.

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 (Willet et al., 1997), 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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