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Maedi-Visna Virus and its Relationship to Human Immunodeficiency Virus

Halldor Thormar
Institute of Biology, University of Iceland, Reykjavik, Iceland

Abstract

Maedi-visna is a slow virus infection of sheep leading to a progressing lymphoproliferative disease which is invariably fatal. It affects multiple organs, but primarily the lungs where it causes interstitial pneumonia (maedi). Infection of the central nervous system was commonly observed in Icelandic sheep (visna), infection of mammary glands (hard udder) in sheep in Europe and the USA, and infection of the joints in sheep in the USA. The name ovine progressive pneumonia (OPP) is commonly used in the USA and ovine lentivirus (OvLV) infection is also a name used for maedi-visna. A related infection of goats, caprine arthritis-encephalitis (CAE), is common in Europe and the USA. The natural transmission of maedi-visna is mostly by the respiratory route, but also to newborn lambs by colostrum and milk. Intrauterine transmission seems to be rare and venereal transmission is not well documented. Macrophages are the major target cells of maedi-visna virus (MVV), but viral replication is greatly restricted in the animal host, apparently due to a posttranscriptional block. The low-grade viral production in infected tissues can explain the slow course of the disease in sheep. The lesions in maedi-visna consist of infiltrates of lymphocytes, plasma cells, and macrophages, and are detectable shortly after experimental transmission. Several studies indicate that the lesions are immune mediated and that cytotoxic T-lymphocytes may be important effector cells. The persistence of the MVV infection is explained by a reservoir of latently infected blood and bone marrow monocytes, which migrate into the target organs and mature into macrophages with proviral DNA transcription, but limited replication of virus. The MVV particles are morphologically similar to those of other retroviruses and the mode of replication follows the same general pattern. The genome organization and gene regulation resembles that of other lentiviruses. In addition to gag, pol and env, MVV has three auxiliary genes (tat, rev and vif), which seem to have similar functions as in other lentiviruses, with a possible exception of the tat gene. A determination of the 9200 nucleotide sequence of the MVV genome shows a close relationship to CAE virus, but limited sequence homology with other lentiviruses, and only in certain conserved domains of the reverse transcriptase and possibly in the surface protein. MVV infection in sheep and HIV-1 infection in humans have a number of features in common such as a long preclinical period following transmission, and a slow development of multiorgan disease with fatal outcome. A brief early acute phase, which is terminated by the immune response, is also an interesting common feature. Like HIV-1, MVV is macrophage tropic and the early stages of the HIV-1 infection which affect the central nervous system and the lungs are in many ways comparable to maedi-visna. In contrast to HIV-1, MVV does not infect T-lymphocytes and does not cause T-cell depletion and immunodeficiency. This is responsible for the difference in the late stages of the HIV-1 and MVV infections and the final clinical outcome. Despite limited sequence homology, certain proteins of MVV and HIV-1 show structural and functional similarities. Studies of MVV may therefore help in the search for new drugs against lentiviruses, including HIV-1. **(AIDS Reviews 2005;7:233-45)**

Key words

Maedi-visna. Ovine lentiviruses. MVV. HIV-1.

Correspondence to:
Halldor Thormar
University of Iceland
Institute of Biology
Sturlugata 7
101 Reykjavik, Iceland
E-mail: halldort@hi.is

Introduction

Visna, which means wasting in Icelandic, is a central nervous system (CNS) disease of sheep that occurred in a restricted area of Iceland from 1935 to 1951. Maedi, meaning shortness of breath, is a respiratory disease of sheep which was found in many parts of Iceland from 1935 to 1965, but it was not fully recognized as distinct from ovine pulmonary adenomatosis until 1939. Both diseases were brought to Iceland with a flock of Karakul rams imported from Halle in Germany in 1933. Maedi and visna were eradicated by an intense slaughtering program lasting from 1944 to 1954. The last cases of maedi were eliminated in 1965[1]. Transmission experiments with both maedi and visna were carried out in sheep by Sigurdsson, et al. from 1951 to 1957[2,3] and showed both diseases to be caused by a transmissible agent, most likely a virus. Based mostly on studies of these two diseases, Sigurdsson introduced the concept of slow viral infections in 1954[4], also comprising ovine pulmonary adenomatosis and scrapie. Visna virus was isolated in cell culture in 1957 from an infected sheep brain which had been kept frozen for a few years, and maedi virus was isolated in 1958 from the lungs of a natural case of maedi. Both viruses were thoroughly studied in the following years and found to be closely related, if not the same virus[5]**.** They have therefore been named maedi-visna virus (MVV), according to their tropism for either the lungs or the CNS.

Although experimental studies of maedi were first done in Iceland, due to the high susceptibility of Icelandic sheep to the disease and consequently its wide distribution and economic impact, the disease had previously been reported in other countries. It was first described as Graaf-Reinet disease in South Africa in 1915[6], as progressive sheep pneumonia or Montana sheep disease in the USA in 1923, where the disease had been known in range sheep since 1915[7], and as la bouhite in France in 1942[8]. In these countries, as was the case in Iceland in the beginning of the maedi epizootic, the disease was not clearly distinguished from pulmonary adenomatosis, which was also widespread in the sheep population. After the pioneering studies of maedi and visna in Iceland, the disease was quickly recognized in sheep in many countries all over the world. Thus, progressive pneumonia of sheep with clinical manifestations of dyspnea, named zwoegerziekte, which was widespread in sheep of the Texel breed in the Netherlands was found to be closely related to maedi[9,10]. Zwoegerziekte had been known in the island of Texel since 1918 and was first described by Koens in 1943 and compared with Montana sheep disease and Graaf-Reinet disease in South Africa[11]. Progressive pneumonia of sheep and goats resembling maedi was also reported in a number of other countries, such as Kenya, India, Kyrgyzstan, Canada, and most European countries[1]. Australia and New Zealand are the only geographic areas where maedi in sheep has never been observed. The disease is widely spread in the sheep population all over North America where it is most prevalent in the Rocky Mountain region, with a seroprevalence of 49%, and least common on the east coast[12]. Maedi was reported in a flock of Merino sheep in East Germany in 1967 and in West Germany in 1970[13]. Notably, maedi was never detected in the breed of Karakul sheep in Germany or elsewhere, despite the fact that the disease was apparently brought to Iceland by imported sheep of this breed. Karakul sheep were first brought to Germany from Astrakhan in Russia in 1901 and still existed in East Germany in 1970. As discussed later, this fact may indicate a different susceptibility to the disease in various breeds of sheep[13].

In contrast to Iceland, where the CNS disease visna became a prominent manifestation of the infection, neurologic signs and CNS pathology are rarely observed in sheep in other countries where progressive pneumonia is prevalent. However, in the Netherlands lesions resembling those of visna were observed in rare cases of sheep with zwoegerziekte[14]. In the USA, meningoencephalomyelitis similar to visna has also been reported as a rare manifestation in ovine progressive pneumonia (OPP), characterized by tremor, ataxia and progressive paralysis of the hind legs[12]. Of 38 naturally infected asymptomatic sheep examined at autopsy, 18% showed subclinical neurologic lesions typical of visna. Thus, the CNS infection is most often subclinical[15]. Rare cases of visna have also been reported elsewhere, e.g. in Germany[16] and in India[17].

Although the Icelandic name of the disease, maedi-visna, has generally been accepted worldwide, OPP is more commonly used in the USA. Ovine lentivirus infection (OvLV) is also used, and the lentivirus infections of sheep and goats have recently been grouped together as small ruminant lentivirus (SRLV) infections[18].

The disease maedi-visna

Maedi was first described in Icelandic sheep in 1952[1,19,20]. Clinical signs appear only in adult sheep, usually more than 3-4 years old. The first signs are

slowly advancing weight loss and shortness of breath, particularly under exertion. Both signs gradually progress and respiration becomes extremely heavy, even at rest. The clinical disease usually lasts for 3-8 months or longer and always leads to death. The disease is non-febrile throughout its course. At autopsy, the lungs show a striking increase in weight, and tracheobronchial and mediastinal lymph nodes are greatly enlarged. The main histologic changes are interstitial inflammation with thickening and infiltration of the interalveolar septa, hyperplasia of smooth muscle fibers and fibrosis. Proliferation of lymphatic tissue is pronounced throughout the lung, with lymphoid follicles within the lung parenchyma being prominent. Epidemiologic studies by Gíslason indicated that maedi is a contagious disease with a very long incubation period of 2-3 years[1,21]. This was later confirmed by transmission experiments[2].

Visna in Icelandic sheep was described by Sigurdsson, et al. in 1957[1,20,22]. This neurologic disease was observed in a limited part of Iceland where maedi was also prominent. It was never observed in sheep less than two years old and occurred mostly as sporadic cases, although on some farms a large number of animals showed signs of visna. It was usually insidious in its onset, beginning with slight ataxia and paresis, particularly in the hind legs. An unnatural position of the head and a fine trembling of the facial muscles were sometimes observed as early signs. The paresis progressed slowly and ended in paraplegia or total paralysis. The disease lasted from a few weeks to several months and was invariably fatal. Sometimes slight intervening remissions occurred. The sheep remained alert to the end. They gradually lost weight, although keeping their appetite and feeding normally. The primary lesion in the brain was found to be meningeal and subependymal infiltration or proliferation of lymphocytes and microglia. Perivascular infiltration was common. A pronounced increase in the number of mononuclear cells in the cerebrospinal fluid (CSF) is a prominent early feature in visna. Demyelination of the white matter of the CNS seemed to occur secondarily and the grey matter was usually unaffected. Lesions were found in the spinal cord in a majority of cases, but only when the brain was also affected. Based on epidemiologic observations in the field and transmission experiments with maedi and visna[2,3], Sigurdsson introduced his concept of slow infections as different from acute and chronic infections. The main characteristics of slow infections are a long incubation period without clinical signs, lasting for months or years and, once clinical signs appear, a fairly regular protracted course ending in death[4]. In addition to maedi and visna, Sigurdsson considered pulmonary adenomatosis of sheep, scrapie, and even some carcinomas and leukemias of animals, to fulfill the criteria of slow infections.

The clinical and pathologic characteristics of visna and maedi in Icelandic sheep, as outlined above, have been reported in sheep in other countries, although clinical signs of visna have rarely been observed outside Iceland[1,12]. In addition to pathologic changes in the lungs and CNS, lymphoplasmacytic mastitis (hard udder) and arthritis have been reported in sheep with OPP in the USA[23,24] and Europe[25], although apparently not observed in Icelandic sheep with maedi. The udder becomes firm and enlarged due to infiltration with lymphocytes and macrophages, which may result in blockage of the teats and failure to properly nurse lambs[12]. In OPP-associated arthritis, one or several joints show synovial hyperplasia with subsynovial lymphocytic and plasma cell infiltration resulting in painful swelling. A disease of goats, which is widespread in Europe and the USA and known as “big knees” because of enlargement of the carpal joints, was demonstrated in 1980 to be related to maedi-visna and caused by a similar virus[26,27]. In addition to arthritis, the clinical manifestations of this disease, named caprine arthritis-encephalitis (CAE), include primary interstitial pneumonia, encephalitis in kids and adults, lymphadenopathy, mastitis, and chronic weight loss, which generally are the same clinical features characteristic for maedi-visna, with the exception of the encephalitis in young animals[18,28].

Routes of transmission

In Iceland, the natural transmission of maedi was mostly by the respiratory route. During the summer, flocks of sheep from large areas used to graze together on common pastures in the highlands and although the disease did not seem to be highly contagious in the open, some transmission apparently took place between individual animals. The housing of sheep together in close quarters during the winter months resulted in a massive spread of infection and this was how the disease mostly spread in Iceland[1]. In the Netherlands, oral transmission of zwoegerziekte to newborn lambs by virus-contaminated milk was observed[10]. Studies from other countries also indicate that air-borne transmission from the respiratory tract and oral transmission by colostrum or milk are the most

common routes of natural transmission of maedi-visna/OPP/SRLV[12,18,28,29]. A recent study has demonstrated viral absorption by intestinal epithelial cells in newborn lambs fed infected colostrum, and passage of virus to mononuclear cells in the underlying lymphoid tissue[30]. Other sources of transmission of OvLV have been studied, but with controversial results. Thus, the occurrence of intrauterine transmission of OPP was reported by Cutlip, et al. in 1981[31]. In a later study, Brodie, et al.[32] observed that most pregnancies in OvLV-infected sheep resulted in uninfected offspring. However, virus was detected by PCR in the blood of 11% of fetuses born to infected ewes and tested prior to colostrum ingestion. A positive correlation was found between the frequency of transmission and young age, presence of viral antigen, and indeterminate antiviral antibody in the mother. In the Netherlands, the success of an eradication program where colostrum-deprived lambs from seropositive dams were reared on bovine colostrum or milk suggests that *in utero* transmission of MVV is rare and therefore of minor importance[33,34]. In a previous study it had been concluded that vertical transmission is of little significance in the epidemiology of the disease[35]. There are few well documented cases of venereal transmission of MVV, and attempts to isolate the virus from semen of infected rams have mostly been unsuccessful. However, rare shedding of OvLV has been reported in the semen of infected rams, particularly associated with a high OvLV load in the lungs and leukocytospermia caused by *Brucella ovis* infection[36]. Other means of transmission of MVV are possible but of minor importance[18].

Maedi-visna is primarily a disease of sheep, but can be transmitted experimentally to goats, and similarly, CAEV can be transmitted to sheep[37]. There is also evidence for natural transmission of these viruses from sheep to goats and vice versa[38]. Species other than sheep and goats were not found to be susceptible to maedi-visna by experimental infection and MVV antibodies have not been detected in humans[39,40]. There is no evidence of transmission of SRLV (i.e. MVV and CAEV) to humans[18].

Breed susceptibility

Early epidemiologic studies in Iceland suggested that certain flocks of Icelandic sheep were more resistant to maedi than others, showing delayed progression of lesions in the lungs[21], and this resistance seemed to be most expressed in crosses between Icelandic ewes and Border Leicester rams. Remarkably, the imported Karakul rams never developed signs of maedi-visna, in contrast to the Icelandic breed, indicating a difference in breed susceptibility. Also in Germany, from where the Karakul sheep were imported to Iceland, they apparently did not show signs of maedi-visna[13]. Studies in the Netherlands, Germany and the USA, where maedi-visna/OPP was studied in different purebred as well as crossbred sheep, have shown a distinct breed susceptibility to the disease rather than to the infection[12,13,41-43]. However, the genetic background of susceptibility or resistance to clinical disease have not been determined in sheep[28].

Control of the disease

The results of programs carried out in various countries in order to eradicate or control the spread of MVV have been recently reviewed[18]. Nowhere except in Iceland has a compulsory program of eradication by slaughter of hundreds of thousands of animals been attempted, since in Iceland the situation was in many ways unique. In the USA, two types of voluntary control measures have been used with good results. In the test-and-cull method, sheep over one year old are tested for OPP virus antibodies and seropositive animals immediately removed from the flock. In the other method, the isolate-and-test method, lambs are removed from seropositive ewes before nursing and are reared in isolation on commercial milk[44]. In the Netherlands, similar voluntary control programs have been successfully carried out[34].

Mucosal immunization by the intratracheal route with an attenuated nonpathogenic MVV clone failed to protect sheep against challenge by a genetically similar, but highly pathogenic, clone of the virus. However, the infection was milder in the immunized compared to control sheep, with fewer virus isolations[45]. Experiments with vaccination against MVV and CAE are ongoing using recombinant vaccinia virus vaccines, attenuated viruses with deletion of selected genes, and DNA vaccines. However, no such vaccines are currently available[28].

An acyclic nucleoside phosphonate analogue, 9-(2-phosphonylmethoxyethyl)adenine (PMEA), which is a potent inhibitor of lentivirus reverse transcriptase (RT) and of viral replication *in vitro,* has been tested as an antiviral drug in sheep inoculated intracerebrally with a highly neurovirulent clone of visna virus. The drug had a marked inhibitory effect on the visna infection if treatment was started on the day of inoculation and continued for six weeks, with fewer virus isolations

and much less inflammatory lesions in the brain. However, if treatment was begun one month after inoculation, only a minor inhibitory effect was found[46,47]. It was concluded that visna virus infection in lambs may be a useful animal model to study drug treatment of lentiviral infections in the CNS[48].

Pathogenesis of maedi-visna

Maedi-visna is a slow infection characterized by a long preclinical period lasting for months or even years[4]. The lesions consist of infiltrates of lymphocytes, plasma cells and macrophages in multiple organs, i.e. lungs, CNS, mammary glands and joints, which gradually lead to clinical signs, most often progressive pneumonia. The inflammatory lesions are detectable shortly after experimental inoculation[49] and increase progressively throughout the course of the infection. In the first transmission experiments with MVV in sheep[50,51], it was found that virus is present in peripheral blood leukocytes within a few weeks after intrapulmonary inoculation, and neutralizing antibodies appear in the serum 2-5 months after inoculation. Complement fixing antibodies appear somewhat earlier[52], and cellular immune response to MVV infection has been demonstrated early in the infection[53-55]. However, the length of time from infection to detectable antibody response can vary greatly and may take many months[12]. There is evidence that sheep experimentally infected with visna virus go through an acute phase of infection during the first weeks or months post inoculation. This is indicated by greatly elevated cell counts in the CSF, reflecting an inflammatory response in the CNS, lasting for a few weeks to months, as well as by virus isolations from CSF and blood leukocytes[49]. Thus, viral isolations from the CSF were most frequent 1-3 months post inoculation, but no isolations were made at seven or nine months. The same observation was made in sheep infected with OvLV where viremia was frequent 2-8 weeks post inoculation, but had declined to low levels by 16 weeks[12]. In an experiment where sheep were inoculated subcutaneously with MVV, infected cells were detected in low numbers after four days in lymph nodes draining the inoculation site. A vigorous immune reaction took place in the lymphoid tissue within 2-3 weeks, with the appearance of plasma cells and virus-specific CD8+ cytotoxic T-cells, and leading to a marked hyperplasia of the lymph nodes. The number of virus-infected cells decreased after the development of specific immune response, suggesting a role for the immune reaction in limiting the acute infection[55].

Although the decline in viral replication is probably caused by the immune reaction during the acute phase of the infection, neutralizing antibodies in high titers and cell-mediated immune response fail to eliminate the virus, and a low grade viral replication and a slowly progressing inflammatory reaction continue relentlessly in the affected organs. Early studies of maedi-visna in sheep showed that the infection is characterized by low levels of free virus in affected tissues, in contrast to the high virus titers observed *in vitro* in cultures of sheep choroid plexus (SCP) fibroblasts inoculated with the MVV. Tissue explants were therefore often necessary to recover the virus. Several later studies demonstrated that, in the infected animals, macrophages are the major target cells of MVV[56-58]. These and other studies demonstrated that, in lymphoid tissues of infected organs such as the lung, virus is almost totally confined to macrophages, of which, however, only a low percentage (~1%) carries the virus. Full replication of virus, with production of free virions, was also found to be greatly restricted in infected cells despite large amounts of viral RNA, apparently due to some posttranscriptional block. The low grade viral production in macrophages in infected tissues can explain the slow course of MVV infection in sheep. However, the restricted viral replication in tissue macrophages and their relatively short life span would lead to an end of the infection without new cells carrying the virus entering the lymphoid tissues from an outside source. Such a source has been found in monocytes, the macrophage precursor cells. Small numbers of monocytes in blood and bone marrow of MVV-infected sheep carry the viral DNA as a provirus with minimum transcription, since only a few copies of viral RNA are found in these cells, in contrast to thousands of copies in mature tissue macrophages. The viral DNA remains latent until the monocytes mature into macrophages in lymphoid tissues of affected organs. Since viral proteins are not expressed in these cells, they can enter the organs by the so-called “Trojan-horse” mechanism without being recognized by the immune system, thus permitting dissemination of the virus[59,60]. The transcriptional block is removed as the monocytes mature into macrophages upon entry into the lymphoid tissues of infected organs. This is apparently caused by cellular transcription factors which bind to binding sites on the promoter of the virus, leading to transcription of the viral genome[61]. The degree of viral gene expression increases with macrophage maturation, but as previously mentioned, viral replication in macrophages is still restricted by a posttranscriptional block. However,

the nature of the mechanisms underlying the various types of replication restrictions in monocytes/macrophages infected with MVV is still poorly understood. The reservoir of latently infected blood and bone marrow monocytes, which differentiate into macrophages as they migrate into lymphoid tissue of the target organs, can explain the persistence of MVV infection in the sheep. Infection of monocytes could be perpetuated by circulation of macrophages producing small amount of free virus, or by replication of latently infected monocyte stem cells[5].

Most studies agree that the monocyte/macrophage is the main or only target cell for MVV. However, visna virus antigen has been observed in fibroblasts and epithelial cells of the SCP in sheep inoculated with a highly neurovirulent strain of visna virus[62], and a later study confirmed the presence of virus in endothelial cells in the CNS, which may facilitate entry of the virus into the nervous system[63]. Other authors have also reported MVV infection in a broad range of cells in the lungs, including type I and II pneumocytes, interstitial and alveolar macrophages, endothelial cells and fibroblast-like cells, and in the mammary gland both epithelial and endothelial cells and fibroblast-like cells[64]. There are somewhat conflicting reports on a possible replication of MVV in lymphocytes, but even if this takes place, it seems to be of low significance[63,65]. On the other hand, it has been reported that MVV is able to replicate in dendritic cells which may be of importance for transfer of the virus to lymphoid tissues[65,66]. Despite intensive studies, the receptors used by MVV for adsorption and entry into host cells have not been identified. MVV has been found to adsorb to and enter a large variety of cells from many different species, although certain cell types, such as Chinese hamster ovary and lung cells, are an exception[67,68]. This is in agreement with a study of the susceptibility of animal and human fibroblasts to visna virus, which caused cell fusion in the monolayer, but very limited virus replication except in bovine cells[69]. The MVV receptors are most likely some common cell membrane molecules, probably proteins, and are not the determining factor in MVV cell tropism, which is more likely determined by cell-specific replication factors[70]. This is supported by a study which showed that OvLV can enter a variety of cell types *in vivo*, although productive infection was restricted to macrophages[71].

The ability of MVV to persist in the infected host in the presence of an active immune response, such as high titers of neutralizing antibodies, has been explained by various mechanisms in addition to the Trojan-horse mechanism. Emergence of antigenic variants in sheep persistently infected with visna virus is well established[72,73] and is believed to play a role in helping the virus to escape neutralization by antibodies. However, other studies of virus isolates in long-term visna showed that, although antigenic variants appear during the course of the infection, they do not necessarily replace the inoculated virus strain, which in some cases was identical to virus isolated from the brain at the time of clinical disease[74,75]. A slow rate of visna virus neutralization by antibodies relative to the rate of virus adsorption to cell surface was observed in an early study[76], and has been suggested as a possible mechanism whereby the virus can spread from cell to cell in the presence of neutralizing antibodies[77]. There is also some evidence for a cell-to-cell spread of infection to cells which are not susceptible to free virus[78]. These mechanisms may further contribute to the persistence of virus in infected animals in the face of an active immune response.

Early studies of Icelandic sheep intracerebrally infected with visna virus indicated that the CNS lesions in visna are immune-mediated, since they were greatly reduced or absent in effectively immunosuppressed animals, whereas viral replication was not affected. It was also concluded that the immune response is directed against visna virus antigens rather than being an autoimmune reaction against CNS antigens[79-81]. Later studies have confirmed the notion that the pathogenesis of the disease is immune mediated. A correlation was found between expression of viral RNA and proteins in infected cells and inflammatory lesions in the brains of sheep with visna[82,83]. Strains of OvLV which replicate rapidly in alveolar macrophages induced lymphoproliferative disease in the lungs, typical of interstitial pneumonia (maedi), whereas less replicative strains were less pathogenic *in vivo*[84]. Later studies of lymphoid interstitial pneumonia demonstrated a correlation between virus load in pulmonary macrophages and the severity of lesions[85,86]. In a study of the immune response in persistent visna virus infection of sheep, it was found that the severity of CNS lesions correlated with the increase in cell-mediated immunity rather than with virus-specific antibodies[53]. It was concluded that the cell-mediated immune response plays a role in the induction of the pathologic lesions in visna, and that CD8+ cytotoxic T-lymphocytes (CTL) may be important effector cells. The active role of these cells in MVV infection is also indicated by the detection of MVV-specific CD8+ cells in efferent lymph and blood of persistently infected sheep[87].

It is likely that once infected macrophages have entered the lymphoid tissue of the target organs and the associated lymph nodes they initiate a low grade viral replication. This induces an inflammatory cascade involving an increase in the number of immune cells, leading to production of lymphokines which stimulate expression of class II major histocompatibility complex (MHC) antigens and proliferation of B and CD8+ lymphocytes. Immune complexes between viral proteins and antibodies may further contribute to the pathologic process which is fed by migration of latently infected monocytes into the tissue, leading to nonspecific damage which terminates in serious disease and death. However, there is not yet a clear understanding of the details of this process[63,88-90].

Biological characteristics of MVV

The first cell culture isolation of visna virus was made in 1957 from the brain of an experimentally infected sheep with advanced clinical signs of visna. The infected brain homogenate was inoculated into cell cultures obtained from the ependyma of a healthy sheep brain. Later, visna virus was isolated from explant cultures of choroid plexus from infected sheep[91]. The cytopathic effect appeared in about three weeks and was characterized by the formation of multinucleated giant cells and stellate cells with increased refractivity. The method of explant culture rather than inoculation of tissue homogenates proved to be more successful in isolation of the virus, and this method was later used to isolate maedi virus from the lungs of sheep with advanced maedi[92]. A comparison of virus strains isolated from visna brains and maedi lungs, respectively, showed them to be closely related although not identical[93]. Thus, both visna and maedi virus strains caused cell fusion with virus particles budding from the cell membrane, forming double-walled spherical bodies, which seemed to undergo a rapid morphologic transition into single-walled virions with an electron-dense, usually eccentric core[94]. However, the replication rate of maedi virus strains was slower and the virus yield smaller than that of visna virus strains in cultures of choroid plexus cells. Visna and maedi virus strains were found to be antigenically related, although antiserum against visna virus failed to neutralize some of the maedi virus strains[94]. It was concluded that the differences between visna and maedi viruses are not greater than those frequently found between strains or types of a viral species[5]. The close relationship between visna and maedi viruses was further established by Gudnadóttir and Pálsson who showed that sheep inoculated intrapulmonarily with either virus developed lesions in both the lungs and the CNS, respectively, characteristic of maedi and visna[50,51]. A comparison of visna and maedi virus to other known animal viruses suggested a relationship with avian and murine oncogenic RNA viruses[5]. The demonstration by Lin and Thormar that visna and maedi virions contain single-stranded RNA and an RT placed the virus firmly in the newly established group of retroviruses[96-98]. In addition to RT, more than 10 other proteins were localized in the virion[99,100] and found to be similar to the structural proteins of other retroviruses[101]. Soon after the isolation of MVV in cell culture, viral agents were isolated by De Boer from the lungs of sheep with zwoegerziekte[10], and by Hadlow, et al. from the lungs of sheep in Montana with OPP[102]. Both agents were found to be closely related to MVV and sera from sheep with either zwoegerziekte or OPP were found to neutralize Icelandic strains of MVV[10,103-106]. These studies also confirmed the resemblance of MVV to the oncogenic RNA viruses. They were therefore grouped together in the family of Retroviridae, characterized by reverse transcription of the viral RNA into double-stranded DNA. However, in contrast to the related oncogenic RNA viruses, MVV was found not to cause tumors in animals[107] or to cause cell transformation in cell cultures[108,109]. The name “lentivirus” was coined for MVV and related retroviruses that cause cytopathic effects *in vitro* and a slowly progressing inflammatory disease in animals, to distinguish them from the oncogenic retroviruses[100]. As in other retroviruses, the lentiviral RNA is reverse transcribed into double-stranded DNA, flanked by identical long terminal repeats (LTR) which contain the promoter for the viral genes. This provirus DNA is transported to the nucleus and incorporated into the cellular genome by the viral integrase[110,111].

The genome and gene regulation of MVV

The complete 9200 nucleotide sequence of the visna virus genome was determined in 1985[112]. In addition to the *gag, pol* and *env* genes, which are characteristic for the Retroviridae family, additional small open reading frames were found separating *pol* and *env*. They were found to represent three genes, later named *tat*, *rev* and *vif*. These and various other auxiliary genes distinguish the lentivirus genus from the oncovirus genus of Retroviridae. In MVV it has been established that the bipartite *rev* gene codes for a regulatory protein

which has the same function as in other lentiviruses, namely to transport un-spliced mRNA from the nucleus to the cytoplasm. This protein, which shows conserved functional organization among lentiviruses, is essential for viral infectivity[113,114]. The protein coded for by the MVV *tat* gene was previously described as a trans-activator protein with a function similar to that of the Tat protein of primate lentiviruses. However, recent work has shown that this protein only upregulates the MVV promoter two to three times as compared to 60-fold for the HIV-1 Tat protein under the same conditions. The CAEV Tat protein was found to be even less active as a trans-activator, and deletion of *tat* had no effect on viral replication. In contrast to the primate lentiviruses, no TAR (Tat activated region) sequence has been identified on the MVV and CAEV LTR and the MVV Tat binds to cellular transcription factors which target the resulting complex to an AP-1 binding site on the LTR. Notably, the basal promoter activity of MVV and CAEV LTR is up to 40-times higher than that of HIV-1 LTR sequences. From this work it was concluded that the MVV LTR promoter is not dependent on Tat protein, and that this protein has a function in MVV different from that in primate lentiviruses[115]. An accessory function of the MVV Tat protein similar to that of the HIV-1 Vpr protein has been suggested, since it is incorporated into the viral particles and induces a G2 arrest in the cell cycle of MVV Tat-transfected cells[116]. However, the biologic role of the MVV Tat protein is still controversial, although it has been established that it is not essential for efficient virus replication[117].

The *vif* gene has been found to be essential for the infectivity of MVV *in vitro* and *in vivo*. Thus, a *vif*-deleted mutant of MVV replicated poorly in cultures of SCP cells, and replication was not detectable in macrophage cultures. The mutant did not cause infection in sheep. An increased mutation frequency was observed in DNA which was reverse transcribed from the *vif*-deleted viral RNA, indicating a function similar to that of the Vif protein of primate lentiviruses – namely to protect the viral genome against damage caused by cellular proteins during reverse transcription[118].

The enzyme dUTPase has been demonstrated in MVV virions and is encoded by a gene located in the *pol* region of the genome. This enzyme, which is absent in the primate lentiviruses, hydrolyzes dUTP to dUMP, and thereby provides a substrate for thymidylate synthase and leads to decreased misincorporation of uracil into DNA. Visna virus and CAEV mutants deficient in dUTPase seem to replicate more slowly in macrophages *in vitro*. Results from *in vivo* studies have shown reduced viral loads in the lungs and possibly other organs of sheep infected with the visna virus mutant. In contrast, in a transmission experiment the mutant was as neuropathogenic in sheep as the wild-type virus. The question of the significance of the dUTPase activity in a natural MVV infection is unresolved[119].

The sequencing of several strains of MVV and CAEV has made it possible to compare these strains with each other and with other lentiviruses. Sonigo, et al.[112] made a comparative analysis of the *pol* gene in visna virus and HIV-1 (LAV) and found a close phylogenetic relationship between the conserved RT and endonuclease/integrase domains of these viruses. Similar results were obtained by molecular hybridization studies of visna virus and HIV-1 (HTLV-III)[120]. Querat, et al.[121] determined the nucleotide sequence of a South African ovine MVV (SA-OMVV) and compared it with an Icelandic strain of visna virus, demonstrating an extensive genetic polymorphism between ovine lentiviruses. The *gag* and *pol* genes were found to be most conserved, particularly the RT region. By comparison of a 200 amino acid-long region of this domain among ovine and primate lentiviruses, a construction of a phylogenetic tree was attempted. Based on the assumption that the time since divergence of the Icelandic visna virus strain K1514 and SA-OMVV was 42 years, it was concluded that radiation of the lentivirus genus is a recent event, the minimal evolutionary time separating ungulate and primate lentiviruses being 430 years. More recently, a phylogenetic tree of the lentiviruses has been established by Wilcox, et al. based on sequence data representing 598 bp of the *pol* gene[122] (Fig. 1). Sequence analysis of a variety of MVV and CAEV field isolates in different countries indicate that the species specificity of these viruses is not as strict as previously thought, since MVV-like viruses have been found in goat populations and vice versa[123]. There are conflicting opinions as to whether the ovine and caprine lentiviruses descended from a common caprine ancestral genotype, or if CAEV descended from an ovine lentivirus adapted to goats[28]. The *env* gene of MVV is highly variable, and although these variants arise mostly by point mutations during reverse transcription, a recent study indicates that recombination occurs in the *env* gene during replication of the virus[124]. The *env* gene of lentiviruses contains conserved as well as variable regions. A sequence similarity has been found between MVV/CAEV surface protein gp135 and the corresponding primate lentivirus gp120 protein in the region between variable loops V2 and V3, indicating that the surface proteins

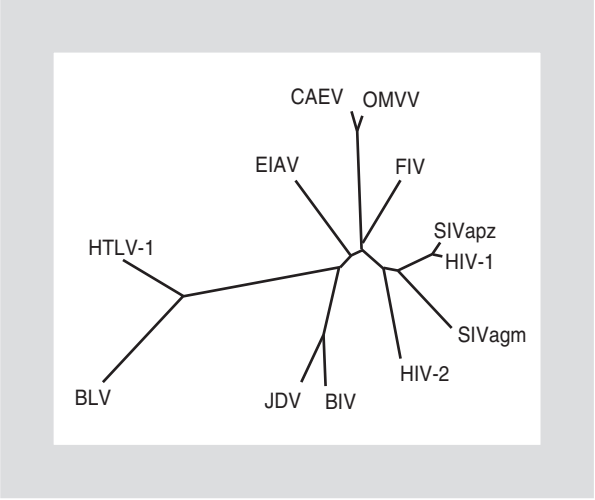

Figure 1. *Phylogenetic tree showing the relationship of 10 lentiviruses. EIAV: equine infectious anemia virus; CAEV: caprine arthritis-encephalitis virus; OMVV: ovine maedi-visna virus; FIV: feline immunodeficiency virus; SIV-cpz: simian immunodeficiency virus of chimpanzees; HIV-1: human immunodeficiency virus type 1; SIV-agm: simian immunodeficiency virus of African green monkeys; HIV-2: human immunodeficiency virus type 2; BIV: bovine immunodeficiency virus; JDV: Jembrana disease virus. The oncoviruses BLV: bovine leukemia virus and HTLV-1: human T lymphotropic virus type 1 are also shown. From Chadwick, et al. J Gen Virol 1995;76:189, with permission of the authors and the Society for General Microbiology.*

of these lentiviruses may have structurally related domains[125].

In addition to elucidating the genetic variation among strains of MVV/CAEV and their relationship to primate lentiviruses, sequencing of MVV genomes has made it possible to study the genetic background for various biologic properties of the virus. In addition to the Rev, Tat, and Vif proteins discussed above, studies have focused on the possible genetic determinants of virulence and of cell and organ tropism. Two different molecular clones of MVV have been studied, namely a clone with high neurovirulence in sheep[126], and a non-virulent clone[127]. These two clones, which differ only by 1% in nucleotide sequence, were studied *in vitro* and *in vivo*. They replicated equally well in SCP cells, but the non-virulent clone LV1-1KS1 replicated significantly less in macrophages and was almost nonpathogenic in sheep in contrast to the neurovirulent clone KV1772[128]. Based on the history of the maedi-visna epizootic in Iceland in 1940-1950, it has been suggested that two variants of the virus were involved, one showing more affinity for the CNS (visna) and the other for the lungs (maedi)[129]. This is supported by transmission experiments in sheep which show that maedi strains isolated from the lungs of sheep with clinical maedi are more pneumotropic and less neurotropic than visna strains isolated from brains of visna-affected sheep. A genetic analysis of a number of strains of either source showed that, whereas there was little variation within the groups of either maedi strains or visna strains, respectively, there was an approximately 11% difference in nucleotide sequence between the groups[129]. A further study of biologic and genetic differences between MVV strains isolated from an infected sheep brain (visna strain) and lungs (maedi strain), respectively, showed that the visna strain replicated more rapidly in SCP cells than the maedi strain, but the replication rate was similar in macrophages. In sheep inoculated intracerebrally, the visna strain induced more severe brain lesions than the maedi strain. There was a 6% difference in the nucleotide sequence of the LTR region of the two strains[130]. A sequence analysis revealed a 53 bp duplication in the enhancer region of the visna strain LTR-U3, which when studied in chimeric viruses appeared to determine the increased tropism for SCP cells and possibly the increased neurovirulence of the visna strain[70]. A number of visna and maedi isolates have been compared and the sequence duplication was almost invariably found in the visna strains and not in the maedi strains[131]. The duplication may be a determining factor in the SCP cell tropism and neurovirulence of MVV, broadening the cell tropism of the virus so that it is able to grow in a variety of cell types, such as endothelial cells of brain capillaries and cells of the choroid plexus, and thus facilitate its crossing of the blood-brain barrier[131].

A comparison of slowly and rapidly replicating MVV isolates suggested a correlation between the promoter activity and the viral replication rate, the slowly replicating strain having a lower basal promoter activity than the rapidly replicating strain and lacking the sequence duplication in the LTR-U3 region[117]. The interaction between *cis*-acting sequences in the LTR and cellular transcription factors seems to regulate MVV replication variably in different types of cells. The Ap-4 binding site in the LTR-U3 region is apparently required for the high basal activity of the promoter, and sequence duplications in the U3 region increase the activity since they are binding sites for key transcriptional factors[132]. In contrast to HIV-1, transactivation by the Tat protein appears to play a minor role in the transcription of MVV genes.

Relationship of maedi-visna to HIV-1 infection

HIV-1 infection in humans and MVV infection in sheep have a number of features in common such as a long

preclinical period following transmission of the virus and a slow development of multiorgan disease which gradually leads to death. Both are, therefore, slow virus infections according to Sigurdsson's definition. Maedi-visna is primarily an immune-mediated lymphoproliferative disease triggered by a persistent low grade viral infection, which gradually leads to severe lesions in the affected tissues and organs. In contrast to HIV, MVV infection does not cause overt immunodeficiency, and MVV does not infect T-lymphocytes, or only to a small extent. The host cell difference between HIV and MVV reflects the difference in receptor specificity between the viruses. Whereas the use of CD4 and chemokine receptors is well established for HIV, the MVV receptor is still unknown, although it seems to be a common cell membrane protein. The major host cells of MVV are of the monocyte/macrophage cell lineage and infection of macrophages is an important feature that MVV shares with HIV-1. MVV infection might therefore serve as a model for macrophage-tropic HIV-1 infection and help elucidate the role it plays in the pathogenic process. This is particularly true for the early stages of HIV-1 infection in which macrophage-tropic virus seems to predominate. Similar to MVV, early targets of HIV-1 infection are the CNS and the lungs where the infection causes lymphoproliferative inflammation, strikingly similar to the lesions characteristic for maedi-visna, which may possibly be immune mediated[133-137]. Like in maedi-visna, monocytes may serve as a reservoir for HIV persistence and may play a role in dissemination of the virus to target organs such as lung and brain. Also similar to visna, infected endothelial cells in the CNS are a potential route for viral entry, either directly or by altering the blood-brain barrier[63,138]. In contrast to maedi-visna, where lymphoid hyperplasia increases throughout the course of the disease and finally leads to death, lymphoid depletion is observed in later stages of HIV-1 infection, due to the predominance of strictly lymphocyte-tropic strains of the virus which lead to depletion of CD4+ T-lymphocytes and to immunodeficiency. This does not occur in maedi-visna, resulting in a difference in the pathologic lesions compared with late HIV-infection. Thus, the difference in T-cell tropism is responsible for the different pathologic manifestations in late stages of these virus infections, and for the difference in the final clinical outcome.

MVV and HIV-1

Although MVV and HIV-1 share limited nucleotide sequence homology, their genomic organization is similar, particularly with respect to the auxiliary genes separating the *pol* and *env* genes[139]. Only three of the six genes found in HIV-1 are present in MVV, and two of these (*rev* and *vif*) seem to encode proteins which are functionally similar to the corresponding HIV-1 proteins. The function of the Tat protein is still questionable – recent studies suggest that it may be primarily an accessory rather than a trans-activating regulatory protein. Another difference in the genomic makeup of MVV and HIV-1 is the presence of a gene for the enzyme dUTPase in the *pol* region of MVV. The biologic and pathogenic significance of this enzyme in MVV infection is still unknown. There is a substantial nucleotide sequence homology in the *pol* genes of MVV and HIV-1, particularly in conserved RT domains[112]. The similarity between the active sites of MVV and HIV-1 RT is reflected in the comparable antiviral activities of nucleoside analogues on replication of the viruses in cell culture[140,141]. On the other hand, several other compounds which inhibit HIV-1 replication *in vitro*, such as TIBO compounds which are non-nucleoside RT inhibitors, a Tat protein inhibitor, protease inhibitors, and bicyclams which are thought to affect chemokine receptors, had no effect on visna virus replication in cell culture[142]. Lectins and sulfated polysaccharides, which also affect adsorption of a number of other enveloped viruses[143], had a minor effect on visna virus. It has been shown that, despite less than 23% sequence homology, there is a distinct structural conservation in the hydrophobic coiled-coil trimer of the transmembrane glycoproteins of HIV-1 and visna virus, which is essential for fusion of the viral envelope with the host cell membrane during viral entry[144]. This is another example of the possibility of using MVV as a model for development of anti-HIV-1 drugs.

Conclusion

Like HIV-1, MVV is macrophage tropic in its host and causes lymphoid hyperplasia in affected organs, lungs and CNS, early in the infection. In the early macrophage-tropic stage, MVV and HIV-1 infections seem to be similar with respect to persistence and pathogenesis. Studies of MVV infection in sheep may therefore help to better understand the early stages of HIV-1 infection. Unlike HIV-1, MVV is not lymphocyte tropic and does not cause depletion of CD4+ T-lymphocytes and immunodeficiency. This explains the marked difference in pathogenesis in the late stages of the two infections and the difference in final clinical outcome.

Despite limited sequence homology, proteins of MVV show structural and functional similarities to proteins of HIV-1. Studies of MVV proteins and how they are affected by various inhibitors may therefore be helpful as a model in the search for new drugs against HIV-1.

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