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MAEDI-VISNA AND RELATED DISEASES

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MAEDI-VISNA AND RELATED DISEASES

1

INTRODUCTION

G. Pétursson and Rikke Hoff-Jørgensen

The concept of slow viral infections was first put forward in 1954 by Dr. Björn Sigurdsson, an Icelandic physician who had been studying some sheep diseases which were introduced into Iceland with the importation of a foreign breed of sheep in 1933. Sigurdsson's main criteria for defining slow infections were a very long initial period without clinical signs lasting months or even years following infection and a rather regular protracted, progressive course, once clinical symptoms had appeared, usually ending in serious disease or death. Sigurdsson included in this list of slow infections maedi-visna, infectious adenomatosis of sheep, scrapie in sheep, Bittner's mammary carcinoma and Gross' leukemia in mice. All of these diseases, except scrapie, are caused by retroviruses.

The characteristics of slow infections as described above are of practical importance for epidemiology, diagnosis and control of these diseases. For many years the slow infections remained primarily a veterinary problem, mainly affecting sheep and goats in certain countries. In recent years, however, the human immunodeficiency virus (HIV) causing acquired immunodeficiency syndrome (AIDS), has suddenly appeared in many countries of the world and brought the slow infection concept forcefully to the attention of the medical profession. The disease problems and the economic effects of slow infections of sheep and goats are increasingly recognized in various countries. For the reasons stated above we feel that this book should be useful for veterinarians and physicians alike.

There is a large group of diseases that would fall into the category of slow infections as defined by Sigurdsson. It has become increasingly evident that this is a heterogenous group. Thus scrapie in sheep together with transmissible mink encephalopathy, dermic coating disease of mule and the human diseases Kuru and Creutzfeldt-Jacob disease are now classified separately as subacute spongiform encephalopathies caused by unconventional agents that as yet have not been characterized in spite of an intensive research

effort.

Another subgroup of agents causing slow infections consists of the lentivirus subgroup of retroviruses. The origin of this name is obvious, lentus meaning slow in Latin. The lentiviruses in contrast to oncoviruses are not oncogenic but cause a variety of inflammatory lesions involving lungs, brain joints, mammary glands, lymphoid tissue and other organs. In this book we have chosen to deal most extensively with the maedi-visna lentivirus infection of sheep, since this is the prototype lentivirus first isolated. For comparative purposes there are also chapters describing a related lentivirus infecting goats, the caprine arthritis-encephalitis virus (CAEV).

The virus of infectious adenomatosis (jaagsiekte) of sheep does not seem to belong to the lentivirus subgroup but rather to the oncovirus subgroup of retroviruses.

The problems posed by lentiviral infections of animals and man are not easily solved. So far no effective preventive measures in the form of vaccines have been found nor any form of therapy that would eliminate the virus from infected animals or humans. It is our hope that this book may be a source of information to those who have to deal with these difficult diseases.

2

MAEDI-VISNA. HISTORY AND CLINICAL DESCRIPTION.

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ABSTRACT

Maedi-visna was accidentally introduced into Iceland by importation of a flock of Karakul sheep intended for breeding purposes in the year 1933. A brief description of maedi-visna in Iceland and its eradication is presented. Owing to the insidious onset of maedi the disease was first recognized in 1939 when it already had spread to many flocks in two different parts of the country. In individual flocks the annual mortality rate was often 15-30 per cent. The heavy losses and the wide spread of the disease are thought to be due to the traditional sheep farming practices in Iceland. An eradication program was started in 1944, slaughtering all sheep in affected areas, and replacing them with healthy young sheep from other parts of the country. The eradication was sucessfully accomplished in the year 1965.

HISTORICAL INTRODUCTION

Maedi which is an Icelandic word meaning laboured breathing is a slow, nonfebrile, progressive interstitial pneumonia of mature sheep characterized by insidious onset, physical weakness, loss of weight and various degrees of tachypnoea and hyperpnoea especially during and following excercise.

Maedi was first described in Iceland in 1939 by G. Gíslason (1) as a progressive pneumonia of adult sheep. It was at that time considered a separate entity, distinct from jaagsiekte of sheep (pulmonary adenomatosis), which was

present in some other parts of the country at the same time (2).

Later these two lung diseases were however found in certain districts occurring in the same flocks, and sometimes even in the same individual sheep. These unexpected findings caused in the beginning of the epizootic in Iceland some confusion and controversy. Most of the affected sheep were also heavily infected with lung worms, which further complicated both clinical and laboratory diagnosis, as long as serological tests or virus isolation techniques were not available.

Maedi and jaagsiekte were both introduced into Iceland by import of some Karakul rams in 1933, and neither disease had ever been recorded before that time in Icelandic sheep. Apparently two rams of the twenty Karakul sheep imported have been latent carriers, one giving rise to an epizootic of maedi, the other one causing epizootic of both maedi and jaagsiekte. These epizootics ravaged in two widely separated parts of the country at the same time. In the flock of Karakul sheep in Germany from which the imported animals originated losses caused by these two lung diseases have not been reported (O. Straub, personal communication).

Due to the long preclinical period and the insidious onset of maedi the disease had spread unnoticed to many flocks when first recognized six years after the importation. Maedi caused annual losses of 15-30% when the disease had taken hold within a flock. A similar insidious and unnoticed spread of maedi, following importations of latent carriers have in recent years been reported in some other countries in Europe, although losses due to maedi are apparently much lower in these countries than observed in Iceland.

A number of slow, progressive pneumonias of adult sheep, with clinical manifestations similar to maedi have been reported in several countries in different breeds of sheep under various names such as ovine progressive pneumonia (OPP) or Montana disease in U.S.A., Graaff-Reinet

disease in S.-Africa, zwoergeziekte in Holland, la bouhite in France etc. Lung diseases of this nature have now been recognized as occurring in most countries of Europe, and several countries in Africa and Asia as well as in N.-America (3).

All these lung diseases of sheep are associated with closely related viruses belonging to the family Retroviridae, subfamily Lentivirinae, which induce progressive deterioration of body condition and laboured respiration reflecting more or less advanced lesions in the lungs, and loss of normal functional alveolar tissue.

The clinical and pathological differences reported by various authors are probably due to slight differences of the virus strains involved and to differences in suseptibility of various sheep breeds affected. Different sheep farming practices in various countries and climates are also likely to influence the severity and course of these lung diseases.

A chronic pneumonia of sheep resembling maedi was apparently first reported by D.T. Mitchell in S.-Africa in the year 1915 (4). Later this disease was described by de Kock in 1929 (5) in sheep at the Graaff-Reinet Experimental Station, hence the name Graaff-Reinet disease. De Kock stressed the histological difference between jaagsiekte (pulmonary adenomatosis) on one hand and the chronic progressive pneumonia on the other hand where heavy lymphoproliferation and secondary hyperplasia of the bronchiolar epithelium were the most prominent lesions.

Marsh described in 1923 a chronic progressive pneumonia of range sheep in the state of Montana (6). In Holland chronic pneumonia of sheep called "zwoegers" was reported already in 1918. Later Koens (7) pointed out the close relationship of zwoegerziekte with Montana sheep disease and the lung disease of sheep described by Mitchell in S.-Africa. In France Lucam in 1942 (8) described a lung disease in certain parts of France which he named "lymphomatose pulmonaire maligne" known by farmers as "la

bouhite" and which he considered to be similar to Montana progressive pneumonia and Graaff-Reinet disease.

In Germany Seffner and Lippman (9) reported slow progressive pneumonia in 1967. Other reports, from Germany have confirmed the serological relationship of this disease with maedi. Hoff-Jørgensen (10) and Krogsrud (11) reported lung diseases of sheep closely resembling maedi in sheep flocks in Denmark and Norway. From various other countries progressive pneumonias of sheep have been reported and found to be closely related to maedi-visna after serological tests and virus isolation techniques became available for comparative studies.

Visna, which in Icelandic means wasting, is a name given to a slow progressive viral encephalomyelitis of adult sheep in Iceland, characterized by weakness especially of the hind legs which gradually progresses to paresis. It was first observed in several flocks in Iceland around 1940 but only in flocks where maedi had already been causing losses for some time. Usually only a few animals in the flock showed signs of visna but in odd flocks losses caused by visna exceeded those of maedi in the same flock. Sheep suffering from visna in the field were also found to be affected with maedi at various stages (12). An association between these two diseases was therefore considered likely from the very beginning. This was later supported in animal experiments. Now maedi and visna are known to be two different manifestations of an infection with the same virus and are therefore referred to as maedi-visna disease (13).

In recent years visna in sheep has been reported in several countries where sheep are raised (10, 14, 15, 16, 17, 18, 19). Usually however only a small number of sheep within a flock show clinical signs of visna.

Some different manifestations apparently associated with maedi-visna virus have recently been reported in a few sheep-raising countries. Thus Oliver et al. reported in 1981 (20) chronic arthritis among mature sheep affected with progressive pneumonia. The carpal and tarsal joints were

most commonly affected, showing swelling of the joints and associated bursae. The affected sheep became lame and emaciated. Ovine progressive pneumonia virus could be recovered from the affected joints, and similar joint lesions were reproduced by inoculation of the virus by various routes (21). It is therefore considered that chronic nonsuppurative arthritis may under certain conditions be one of the manifestations caused by the ovine pneumonia virus.

Chronic nonfebrile mastitis affecting sheep suffering from ovine progressive pneumonia was first reported by Cross et al. in 1975 (22) and they suggested that the lesions found in the udder were caused by ovine progressive pneumonia virus. Similar lesions were described by Griem and Weinhold in 1976 (23) in German sheep. Oliver et al. in 1981 (20), studying naturally occurring progressive pneumonia of sheep of mixed breed, found some of these sheep affected with chronic indurative mastitis with massive lymphoid proliferation. Chronic indurative mastitis in Texel-sheep associated with maedi-visna virus infection was described by van der Molen et al., in 1985 (24). The mastitis resulted in reduced milk production and retarded growth of lambs from affected dams.

These manifestations may however be present more often in certain breeds of sheep infected with maedi-visna virus than observed in previous studies where the main objects of study have been lesions affecting the lungs and the central nervous system.

In Iceland, where maedi was introduced into a very susceptible virgin population of sheep, it soon became evident that maedi was a contagious disease. In retrospective studies carried out in the field by Gíslason (1) it was often possible to trace the spread of maedi into healthy sheep flocks following purchase of apparently healthy animals from infected flocks. However the disease was usually first recognized clinically in the flock 5-6 years after the introduction of the animal that carried the

infection into the flock. Losses due to maedi then increased rapidly in the flock and 10 years after the introduction of the disease the annual mortality rate was often 15-30%, which appears to be much higher than recorded in other countries. Based on records from the field the preclinical period of maedi was estimated to be at least 2-3 years. Maedi could be transmitted to healthy sheep by direct contact, by injecting material from infected organs intranasally, intrapulmonary and intravenously, and by contaminating the drinking water (25). Indirect spread of maedi in Iceland appeared to be exceptionally rare (3).

When tissue culture techniques became easily available virus was isolated from lungs affected with maedi in 1958 (26). Later maedi could be produced by inoculation of this virus into healthy sheep by various routes, and the virus again consistently recovered from these sheep (27, 28, 29).

Visna was first transmitted by intracerebral inoculation using filtered material from brains and spinal cords of natural cases of visna. By serial inoculations the infective material was maintained in experimental animals after the disease had been eradicated from all sheep farms in the country. Later the causative virus of visna was isolated in tissue culture and was found to produce visna when inoculated into healthy sheep.

CLINICAL FEATURES

Maedi

Clinical signs of maedi are only observed in adult sheep, usually in sheep more than 3-4 years old, and often the disease is first seen in the pregnant or nursing ewes. The animals loose condition, and the respiration becomes laboured, which at the beginning is only apparent when the sheep have been exposed to some strain. The respiration rate becomes very rapid, sometimes excessively high, 80-120/min. In uncomplicated cases of maedi the body temperature and the pulse rate remains within the normal range.

As the disease progresses the respiration becomes more and more laboured. Sometimes the nostrils are dilated and flank breathing or pumping aided by the abdominal muscles is often seen. At this stage the laboured respiration is accompanied by characteristic rythmic jerks of the head, and sometimes dry coughing. In order to reveal the disease in its early stages it was the practice of some farmers to expose their flock to physical strain in various ways in the autumn. Those sheep that showed laboured respiration for an abnormal length of time after such strain were disposed of as they were considered poor risk not fit enough to give birth to a lamb and rear it the following summer. Ewes affected with maedi often give birth to small and weak lambs and the milk yield is apparently decreased. It is doubtful whether recoveries ever occur after maedi-visna has become clinically evident.

After the disease has reached the clinical stage, the sheep can be expected to survive for 3-8 months, but in a few cases the course of the clinical disease can be considerably longer, even one or two years if the animal is never exposed to any stress. An anaemia of hypochromic type is often observed in advanced cases. Prolonged leucocytosis of lymphocytic type is also often found. Under field conditions sheep suffering from maedi-visna usually succumb to an acute bacterial pneumonia.

Due to the extremely slow progress of the disease there will be a certain number of infected sheep in the flock that do not show clinical evidence of maedi during their limited lifespan although some maedi lesions are present and found at post-mortem examination.

Visna

Visna is very insidious in its onset, and under field conditions only mature sheep seem to become affected. The first sign is often noticed because the sheep lags behind the flock when driven, particularly when the ground is uneven or steep. Gradually weakness of the hind legs, abnormal posture and stumbling becomes apparent. Later the

power to extend the fetlocks becomes impaired, and affected animals are seen resting the end of the metatarsus on the ground. Sometimes the head is tilted a little to one side, and a fine trembling of the lips and facial muscles is seen.

The paresis of the limbs progresses slowly; the sheep prefers to lie down even when grazing. Finally the animal becomes paralytic and cannot get on its feet. The animal appears to be alert to its surroundings and uptake of food and appetite is normal. The course of the clinical disease is protracted for several months, even a year, before the stage of total paralysis is reached. Under farming conditions affected animals were disposed of when the clinical signs became evident.

Clinical laboratory tests have shown elevated gammaglobulin levels in cerebrospinal fluid (12). There is also an increased number of mononuclear cells in the cerebrospinal fluid, varying from from 40 up to 2000 cells per mm^3. Body temperature remains in uncomplicated cases within normal limits.

Other clinical manifestations

Some recent reports indicate that the mammary glands are perhaps most susceptible to injury by the ovine progressive pneumonia virus and by the virus of zwoegerziekte. As the clinical manifestations of the udder are of the chronic type they are often overlooked by the sheep farmer. The effect on the udder of the dam is often first noticed when the offspring shows an unusually poor growth rate. The signs are inconclusive. A bilateral diffuse, indolent induration, sometimes with firm nodules in the ventral part of the udders, are often the only clinical evidence. The accessory lymph nodes are only slightly enlarged, and the milk has a normal appearance (22,24).

A chronic nonsuppurative arthritis has been described as a manifestation sometimes associated with ovine progressive pneumonia. The carpal and tarsal joints are most commonly affected, often bilaterally. The joints are swollen, indolent and the joint capsules and synovial

membrane are thick, with marked periarticular fibrosis. Various degrees of lameness are the results of these severe joint lesions, and usually the animals are found to be in poor physical condition as well. Arthritis of this type seems to be refractory to treatment.

ERADICATION OF MAEDI AND VISNA IN ICELAND

Maedi-visna caused heavy losses in Iceland in the 1930's and 1940's. When the disease had taken firm hold within a flock the majority of the ewes succumbed to maedi-visna when 4 to 7 years old, whereas the normal productive lifespan of ewes in non-affected flocks was 8 to 10 years. Certain strains within the Icelandic breed were apparently more resistant than others i.e. the lesions progressed more slowly. Rams of such strains were therefore in great demand for breeding purposes, in order to prolong the life of the offspring.

Various therapeutic measures have been tried in order to cure or delay the progress of maedi-visna. All such efforts have given inconclusive or negative results. Spread of maedi-visna can be arrested by preventing close contact between healthy and affected animals by the use of fences or other appropriate measures.

In Iceland where losses due to maedi-visna became so heavy in certain districts, that sheep farming became an economically hopeless task, eradication was attempted by slaughtering all sheep on every farm within affected areas, and restocking the farms with sheep from non-affected districts. Due to financial restrictions and limited number of sheep available for restocking the eradication program had to be stretched over a period of ten years (1944 to 1953).

Maedi reappeared several times in a few of the restocked farms, and could in all cases but one, be traced back to contacts with affected animals. The eradication of the disease was finally accomplished with success in the year 1965. This very drastic and expensive stamping out

program was only justified because of the exceptional heavy losses the disease caused in Iceland. It is doubtful if such drastic control measures will ever be attempted in countries where the disease is endemic and the losses caused by maedi-visna are much lower than experienced in Iceland.

Due to climatic conditions sheep farmers in Iceland have to house and feed their sheep throughout the winter. During the summer months the sheep graze on common hill and mountain pastures where sheep from different farms, or even from different parts of the country, roam freely without any

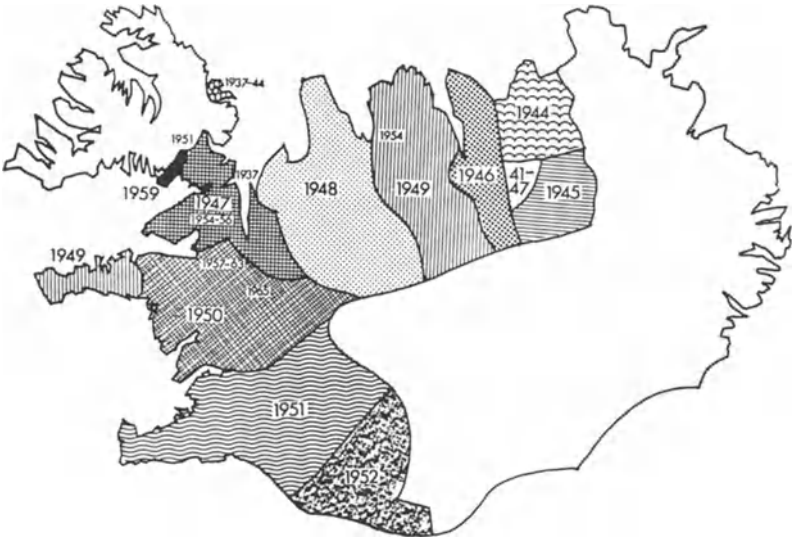

Fig. 1. A map of Iceland showing the different quarantine areas, which had been established when the program to eradicate maedi-visna and jaagsiekte was initiated in the year 1944. The large letters indicate the years when the slaughtering of all sheep within the quarantine area was carried out. Recurrence of maedi in a few places necessitated slaughtering of a limited number of flocks in the years 1951-1965 (small letters). Experimental eradication, which was carried out on a few flocks in the years 1937-1943, is also indicated with small letters.

shepherding or supervision. In the autumn all sheep from the common pastures are rounded up and brought to big sorting pens where they are reclaimed by the various owners. It is evident that a disease that is primarily transmitted by the respiratory route and has an extremely long preclinical course is difficult to control under sheep farming practices of this kind.

When it was well established that a previously unknown contagious lung disease had been introduced into the country and taken hold in a number of sheep flocks causing heavy losses, various measures were instituted to halt its spread. However at that time the nature of the disease and its very insidious onset were not fully understood so not all of these attempts were equally effective.

The main emphasis was put on preventing free movement of sheep from affected flocks or areas. Hundreds of miles of barbed wire fences were erected for that purpose and guards were continuously at control work to keep sheep from breaking through these fences and thus coming in contact with sheep from unaffected areas. Sheep that crossed these quarantine fences were ruthlessly slaughtered. Some fences built in the beginning were later found not to serve a useful purpose since the disease had already been incubating in flocks behind the fences, when they were erected, and the disease became clinically evident one or two years later.

Although some of the first attempts to arrest the spread of the disease failed, later on when more experience had been gained and epidemiological data collected regarding the insidious nature of maedi, the control measure became more effective and further spread could be prevented in most cases. By that time, however, the disease had spread to more than half of the sheep-producing districts of the country. More and more sheep flocks within the affected districts became affected and the annual losses caused by maedi often were so heavy that sheep farming became economically hopeless. The government therefore decided to

combat maedi-visna by slaughtering all sheep on every farm, affected or not affected within those parts of the country where the diseases maedi and jaagsiekte had taken hold, and restock them with young healthy sheep from non-affected districts. In order to enforce such drastic control measures a special Act of Parliament to this effect had to be adopted.

Due to the limited number of young healthy sheep available each year for this purpose it was from the beginning evident that such a program of eradication was bound to take several years to accomplish. When this program was started in 1944 the spread of the lung disease based on intensive epidemiological studies had been well established and the affected areas were already securely fenced in, forming a number of quarantine areas.

Each autumn during the period 1944-1953 all sheep within one or more of these quarantine areas were slaughtered, and then restocked either the same autumn or the following autumn. In areas where scrapie or paratuberculosis were endemic, the areas were kept free of all sheep for at least one year in an attempt to get rid of these two diseases as well. The slaughtering and restocking had to be carried out in the months of September and October each year due to various local reasons.

Usually no proper disinfection was carried out on the farms, but the premises and sheep sheds were kept free from sheep at least for three months prior to restocking. In certain quarantine areas maedi reappeared several times on farms some years after restocking. Epidemiological studies of these recurrences of maedi on restocked farms revealed that in all cases except one they could be traced back to accidental contact with affected sheep, owing to the long and silent incubation period of maedi, and the trend to speed up the restocking program. Only in one case was the recurrence due to indirect transmission, apparently from affected lungs used to feed foxes. All sheep on these farms and in-contact sheep were consequently slaughtered. The

last recurrence of maedi on a restocked farm occurred in the year 1965.

Although the eradication of maedi and jaagsiekte in Iceland was successful the program failed to eradicate scrapie and paratuberculosis of sheep as intended at the beginning. Jaagsiekte was eradicated in the year 1952 and the last clinical case of visna was seen in the year 1951.

It should also be underlined that the decision to use such very drastic measures to eradicate primarily maedi and jaagsiekte was by no means unanimously supported in the beginning when the understanding of the nature of these diseases was rather limited. It can therefore be described as a bold and optimistic attempt to save sheep farming in the country, which at that time was of great economical importance.

It has been estimated that more than 105,000 sheep succumbed to maedi, and more than 650,000 sheep had to be slaughtered in order to eradicate the disease. The campaign lasted for almost 30 years followed by various control measures and control tests for several more years. The eradication scheme and various control measures were directed and supervised by a board of five members elected by the Parliament as stipulated in the law. Within each quarantine area all sheep owners had to form an association which was responsible for the slaughtering and restocking according to certain regulations.

In this way the sheepowners themselves became legally responsible for the task of gathering and slaughtering every single sheep of the old stock within the quarantine area. This difficult task had to be accomplished before a certain date in the autumn, after which the restocking began. These associations of sheep owners were also given the duty to select, purchase, transport and distribute the new young stock to the numerous persons entitled to acquire these sheep.

In this way sufficient manpower familiar with local conditions and sheep farming was secured for this important

task, and the success of the eradication scheme was to a great part due to the loyalty of Icelandic farmers to the drastic eradication measure instituted.

For several years following restocking regular examination of sheep lungs from restocked farms were carried out in the abattoirs, blood samples were collected and tested for presence of antibodies against maedi virus in order to detect possible recurrences of maedi in the restocked districts.

Iceland is apparently the first country that has succeeded in getting rid of a slow viral disease in the national stock of sheep.

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3

MAEDI - VISNA . PATHOLOGY AND PATHOGENESIS

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ABSTRACT

Infection of sheep with maedi-visna virus (MVV) causes a multi-organ disease, i.e. encephalitis, pneumonia, mastitis and arthritis. Both the spectrum of organ changes and the character of pathological lesions are similar to that observed in the related lentiviral infection of goats. The tissue tropism of infection with human immunodeficiency virus (HIV) is partly similar and the pathological lesions, especially of the lungs of pediatric patients with AIDS, resemble maedi in sheep. There is a difference in the susceptibility of different breeds of sheep and a variation in the biology of different strains of MVV, reflected in the incidence of different organ manifestations of the infection in various breeds. Thus arthritis has only been observed in American sheep infected with progressive pneumonia virus (PVV) and Icelandic sheep seem to be exceptionally susceptible to encephalitis. A viremia, which develops shortly after infection, apparently plays a major role in the spread of the infection. In American breeds of sheep the main targets of infection are macrophages and they seem to play a key role in development of lesions, whereas in Icelandic sheep a wide variety of cells are permissive for infection with the MVV, among them lymphocytes and glial cells. Early lesions have been shown to be immune-mediated, the damaging immune response being directed against virus-induced antigens. A change in the pathogenetic mechanisms may, however, occur with time, as indicated by the appearance of plaques of primary demyelination many years

after infection, resembling lesions characteristic of multiple sclerosis.

INTRODUCTION

There is increasing evidence that infection with the maedi-visna virus (MVV) causes a multisystem disease. The best known organ manifestation of infection with the MVV is a chronic interstitial pneumonia, first reported in 1915 in South Africa (1) and described in detail by Marsh in the early thirties (2). Consequently the pulmonary affection has been reported in many countries under several different local terms (3, see also chapter by Houwers). In Iceland the lung affection was first diagnosed in 1939 (4), six years after import of Karakul sheep, which were healthy carriers. It was given the Icelandic name maedi, i.e. dyspnoe.

Another organ manifestation of MVV infection, a meningoencephalomyelitis of sheep, was first recognized in Iceland (5) and named visna, meaning wasting. The causative agent was first isolated from the brain of visna-affected sheep (6) and few years later virus was also isolated from maedi lungs (7). Shortly thereafter it was shown that maedi and visna were different organ manifestations of infection with the same virus (8,9,10). In general the primary manifestation of MVV infection has been the pulmonary affection. The epizootic in Iceland was exceptional, in that the central nervous system disease (CNS) was the main cause of morbidity and death in some flocks (3), indicating emergence of a more neurotropic strain of virus in the field.

More recently it has been shown that besides the traditionally well known organ manifestations of the MVV infection, i.e. pneumonia and encephalitis, other organs may be affected. Thus the mammary gland seems to be one of the main target organs of the virus (11,12,13,14,15,16) and in addition to airborne spread, infection of suckling lambs via

colostrum and milk is important in spreading the infection (see chapter by Houwers).

In the United States there is evidence that the MVV or progressive pneumonia virus variously called PPV or OPPV (from ovine progressive pneumonia) may also cause an arthritis (13,17,18), especially of the carpal joints. This has not been described in other countries and the same applies to vasculitis described by Cutlip et al. (19).

Other organ manifestations of this systemic infection with MVV have been described occasionally but definite evidence for a causal association is lacking.

In the following description of the pathological manifestations of the MVV infection the emphasis is on those changes which are best known and have most frequently been reported in infections with MVV in various countries, i.e. the chronic interstitial pneumonia and the meningoencephalomyelitis. Furthermore, the lesions of the mammary gland will be described and finally the arthritis caused by the PPV in the United States will be dealt with, although at the present stage of knowledge it seems to be strain and/or host dependent.

PATHOLOGY

Maedi

Most of the original reports on the pathological lesions of maedi were isolated observations made before the causative agent was known and the diagnosis could therefore not be confirmed by either serological tests or virus isolation. The following description of the lungs in maedi is mainly based on more recent detailed pathological studies on cases where the diagnosis has been confirmed by either serological tests and/or virus isolation in individual cases or in the herds examined (12,13,20,21,22,23,24).

Macroscopic changes. In fullblown typical cases the lungs are diffusely affected, voluminous and do not collapse

when the thorax is opened. Sometimes impressions of the ribs can be detected. The weight is frequently two or three times the normal weight, at least when compared with age-matched controls relatively free of worm infestations (23). The pleura is in general smooth and glistening. Occasional greyish, dull areas indicating a healed pleuritis are found, probably due to some superimposed infection. An exudate is not found in the pleural cavity. The consistency of the lungs is firm, fleshy or rubbery. The colour is grey-reddish or grey-brown. Both the pleura and the cut surface is frequently studded with minute, 0.5 to 1.0 mm, grey or grey-yellowish foci. Such foci, scattered through the lungs with normal or emphysematous lung tissue in between may be the initial changes observed (21). The cut surface is relatively dry. In early stages the anterior borders of the apical, cardiac and diaphragmatic lobes are affected. In some series, multiple sharply demarcated dark-red areas of consolidation, seem to be the prevalent macroscopic finding (25), which may pose a differential diagnostic problem as such changes are similar to those observed in secondary bacterial bronchopneumonia, a common complication in maedi. The occurrence of such a complication is probably not fortuitous, but is probably due to lowered resistance of a maedi-affected lung to bacterial infection (22) and is apparently a common cause of death. An enlargement of mediastinal and tracheo-bronchial lymph nodes is usually observed and may be considerable.

Microscopic changes. One of the most characteristic histological changes is proliferation of lymphoid tissue with formation of regular lymph follicles, frequently with active germinal centers (Fig.1). These lymph follicles are usually either perivascular or peribronchial in localization but do sometimes not show any relation to these structures, which may be due to the plane of section. In a semiquantitative study Cutlip et al. (22,26) found lymphoid proliferation to be the most constant feature found in maedi and in Wandera's series (21) lymphoid hyperpasia was

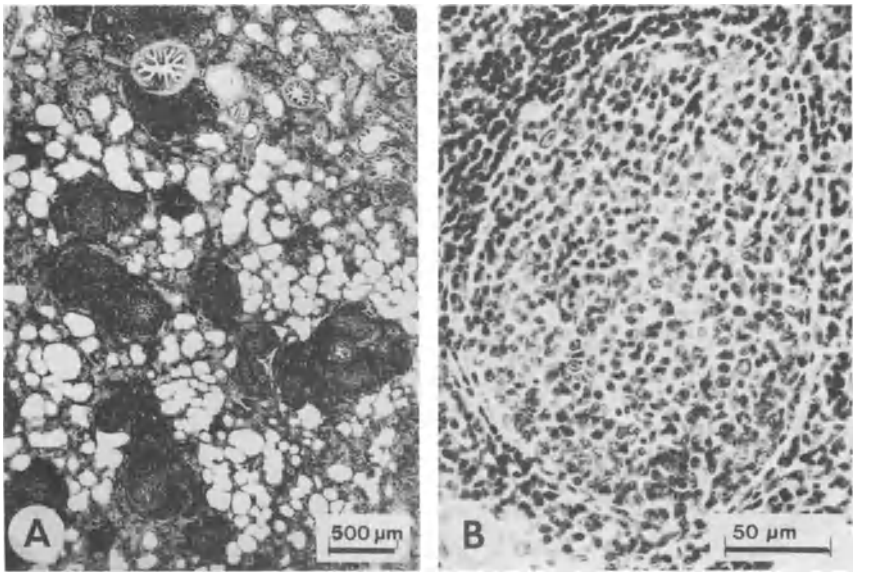

Fig.1. A) Pronounced lymphoid hyperplasia with numerous partly peribronchial lymph follicles.H.E. B) Lymph follicle in the lung with active germinal center.H.E.

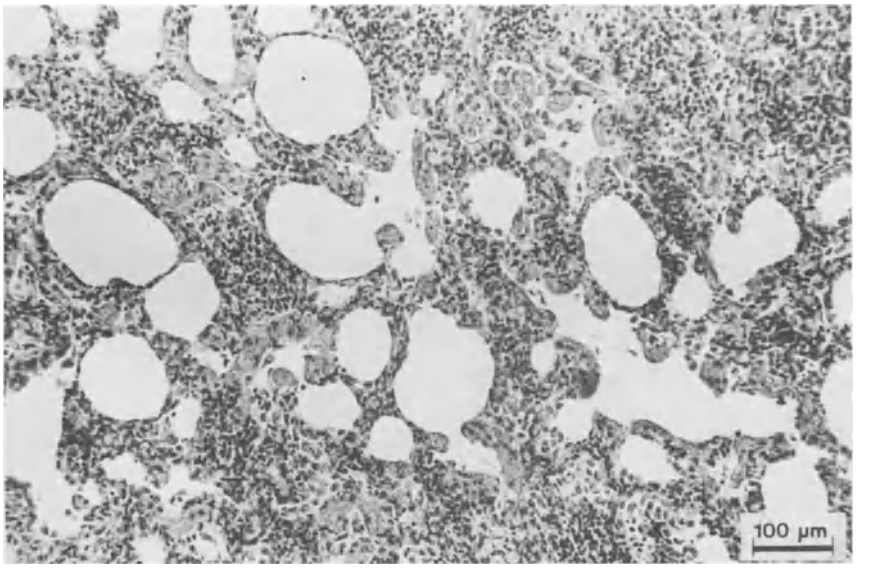

Fig.2. Diffuse interstitial inflammation with thickening of interalveolar septa.H.E.

prominent in about 2/3 of the lungs examined.

According to studies on young lambs of maedi-affected ewes (11) and transmission experiments (27,28,29,30) the initial changes are apparently small perivascular or peribronchial accumulations of lymphoid cells sometimes accompanied by microfoci of desquamative pneumonia.

Another important characteristic of the pathological lesions in maedi is an inflammatory infiltration of the interalveolar septa, which are thickened. This may lead to narrowing and in some areas to almost total obliteration of the alveolar spaces (Fig.2). The interstitial inflammation may evolve early after infection concomitantly with the lymphoid proliferation (11,30). In a certain proportion of cases the interstitial inflammation may be very prominent without a proportional increase in lymphoid tissue (21,22, 23). The inflammatory infiltrates consist primarily of lymphocytes and macrophages with some admixture of plasma cells whereas polymorphs are not observed in uncomplicated cases (Fig.3).

There is an increase of reticular fibres (Fig.4), which contributes to the thickening of the interalveolar septa (23,24). In some series a conspicuous fibrosis has been observed in far advanced cases (12,20), which occasionally is very pronounced and may take on a embryonal or myxomatous pattern (22). However, the extent of mature fibrosis varies and can be slight even in far advanced stages (21,23,24). Elastic fibres on the other hand seem to be fewer and often fragmented, which may contribute to the loss of elastic recoil of the lungs (12,20,23,24).

Hyperplasia of smooth muscles is a common feature. This hyperplasia is found at different levels, but is often prominent in the alveolar ducts (23,24), leading to knob-like thickenings at the openings into alveoli (Fig.4), but is also observed in bronchiolar and bronchial musculature (20).

The alveolar epithelium may take on a cuboidal form,

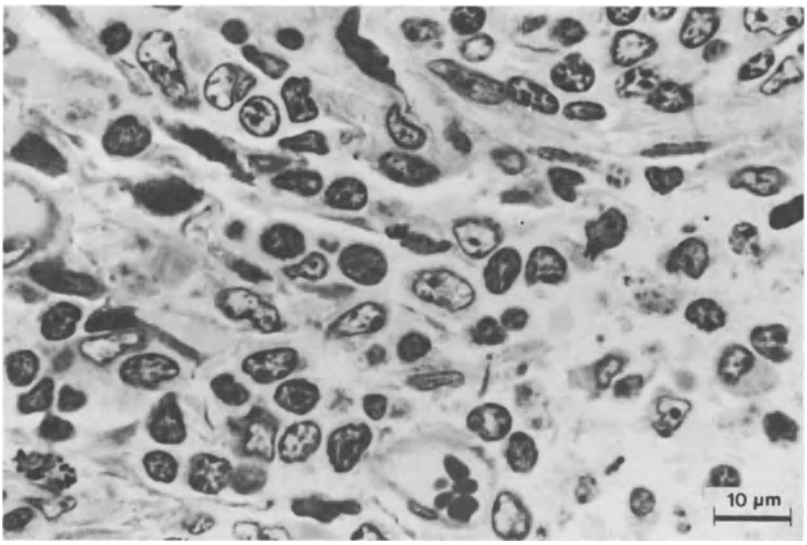

Fig.3. Inflammatory infiltrate in interalveolar septa, consisting of macrophages, lymphocytes and a few plasma cells.Toluidine blue.

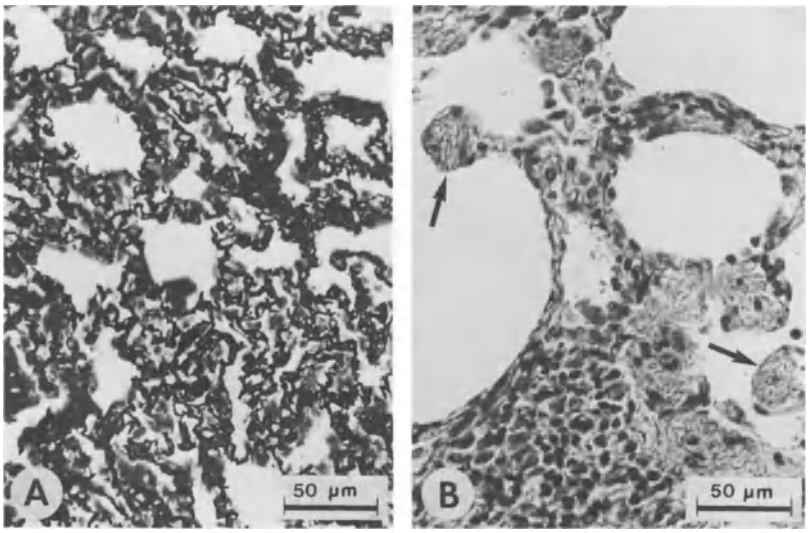

Fig.4. A) Increase of reticular fibres in interalveolar septa. Gömöri B) Hyperplasia of smooth muscles with knob-like thickenings at the tips of interalveolar septa (arrows).H.E.

so-called epithelization (Fig.5). According to an ultrastructural study (31) this represents hyperplasia of granular pneumocytes. The epithelization is often prominent in areas where thickening and fibrosis of the interalveolar septa is pronounced and may be accompanied by disorganization of the lung tissue (Fig.5). Such areas may bear a superficial resemblance to pulmonary adenomatosis. The epithelium of bronchi and bronchioles is often hyperplastic (Fig.6) with increase in mucous-producing goblet cells. Occasionally inflammatory polyps, covered by hyperplastic epithelium (Fig.6), extending into bronchial lumen have been observed (20,23).

Alveolar exudate is generally not conspicuous, except in cases with superimposed bacterial infection. In uncomplicated cases it consists of desquamated macrophages, which sometimes fuse to form bi-or tri-nucleated cells. Multinucleated cells similar to those typical for the cytopathic effect of the MVV in vitro (Fig.6) appear to be rare. They have, however, occasionally been observed in the alveoli (21,24,32) and in the interstitium (33).

Vascular changes, indicative of a pulmonary hypertension, i.e. thickening of the media of pulmonary arteries and reduplication and splitting of the elastic laminae, have been described (20,23,24) and hypertrophy of the right heart has been observed (20). A rare finding is an arteritis of small and medium-sized arteries (20) similar to the more systemic vasculitis described in infection with PPV in the United States (19).

Another constant feature observed in maedi is a hyperplasia of the mediastinal and tracheobronchial lymph nodes. Both cortical and paracortical areas are enlarged but the hyperplasia of the cortex is according to a morphometric study distinctly more pronounced (34).

The overall pathological picture observed in full-blown cases of maedi, with lymphoid proliferation, chronic interstitial inflammation and muscular hyperplasia combined with a varying degree of fibrosis, is characteristic. The

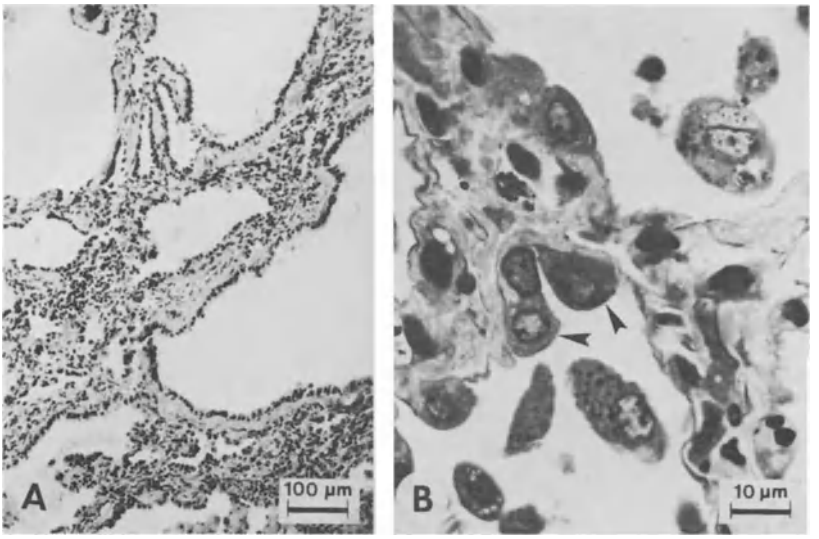

Fig.5. A) Fibrotic, disorganized area with cyst-like cavities lined by cuboidal cells.Masson-Trichrome. B) Hyperplasia of alveolar epithelium(arrowheads). Desquamated macrophages in alveoli.Toluidine blue.

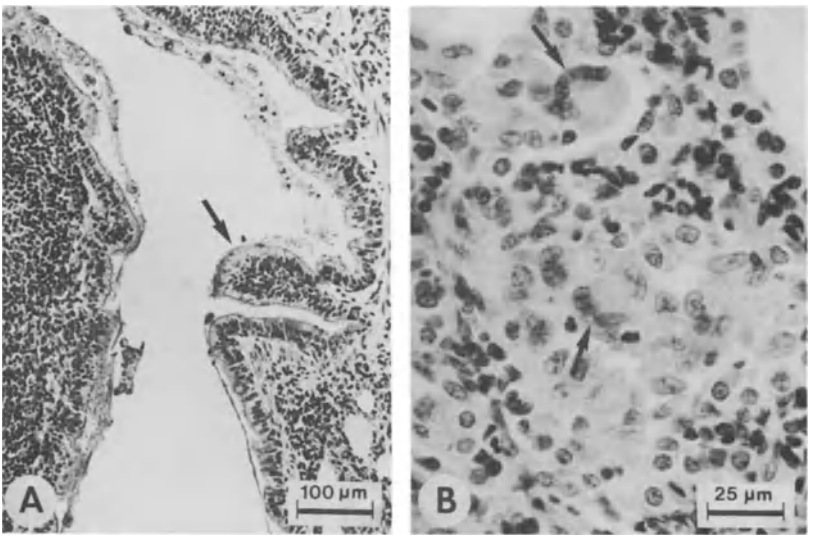

Fig.6. A) Hyperplastic epithelium and inflammatory polyp in a bronchiole(arrow). Lymph follicle to the left.H.E. B) Multi-nucleated giant cells(arrows).H.E.

different features, however, of the histological changes are not pathognomonic for the MVV infection. Lymphoid proliferation can be provoked in the lungs with various irritating substances or agents (35) and is a prominent feature of mycoplasma pneumonia of sheep (36). Worm infestation provokes a prominent muscular hyperplasia (37) and hyperplasia of the alveolar epithelium, i.e. so-called epithelization, may represent a non-specific response to the thickening of the interalveolar septa (38) and is a frequent finding in pneumonias of sheep (39).

Visna

Shortly after visna had been detected and shown to be transmissible by intracerebral inoculation (5) Gudnadóttir and Pálsson (40) succeeded in transmitting visna by intrapulmonary inoculation of the virus. Thus by mimicking the natural route of infection they provided evidence, that the CNS disease was but an additional manifestation of an infection with MVV, which was consistent with the experience in the field. Visna always occurred along with maedi during the epizootic in Iceland (3) and was with the above mentioned exception usually subclinical which also seems to be the general rule in other countries.

Visna has now been reported in several breeds of sheep in many countries (13,20,21,22,33,41,42,43,44,45, 46,47) and has, with possibly one exception (47), always been detected in conjunction with maedi-lesions in the lungs. A few studies have been done to obtain information on the incidence of the CNS lesions and they indicate that approximately 10 per cent of sheep with maedi also have visna (21,22,46).

The pathological lesions show a similar pattern in the various breeds of sheep and are, as reported by Sigurðsson and co-workers (5,48), comparable in natural and experimentally transmitted cases, regardless of the route of infection (29,40,49,50,51).

Clinical cases of visna have not been observed in Iceland since 1951 (3) but the disease has been studied extensively in transmission experiments in our laboratory ever since it was detected. The following description of the pathological changes will mainly be based on results obtained in a series of experiments, which were started some 15 years ago, using intracerebral inoculation of the same strain of virus (K1514). We have studied the CNS lesions in more than 100 sheep, using the same 9 standard planes of sections from the brain, three different levels of the spinal cord (and occasionally the entire spinal cord has been studied), the sciatic and optic nerves and retina. Furthermore the cellular exudate in the spinal fluid has been studied. The lesions evolving after experimental transmission have been analysed from two weeks to 11 years after infection at different ages, i.e. from foetal to adult sheep. The description of the pathological lesions has been a subject of several reports (24,52,53,54,55, 56,57).

Macroscopic changes. In general the brain and spinal cord appears normal on macroscopic examination. Rarely the leptomeninges over the brain and spinal cord may show areas were the meninges are not translucent but greyish and apparently thickened. The choroid plexus sometimes shows granular thickenings and on some occasions with very extensive lesions, the white matter of cerebrum, brain stem and cerebellum may appear softened and show a greyish discoloration. On a few occasions we have observed relatively sharply demarcated greyish plaques in the white columns of the spinal cord.

Microscopic changes. An inflammatory infiltration of the leptomeninges of the brain is a very common feature and does frequently extend to the spinal meninges.It is usually not diffuse but tends to be accentuated over the superior frontal gyrus, hippocampic fissure, pyriform (Fig.7) and occipital lobe as well as the cerebellar lingula. In the spinal cord it is usually most marked over the anterior

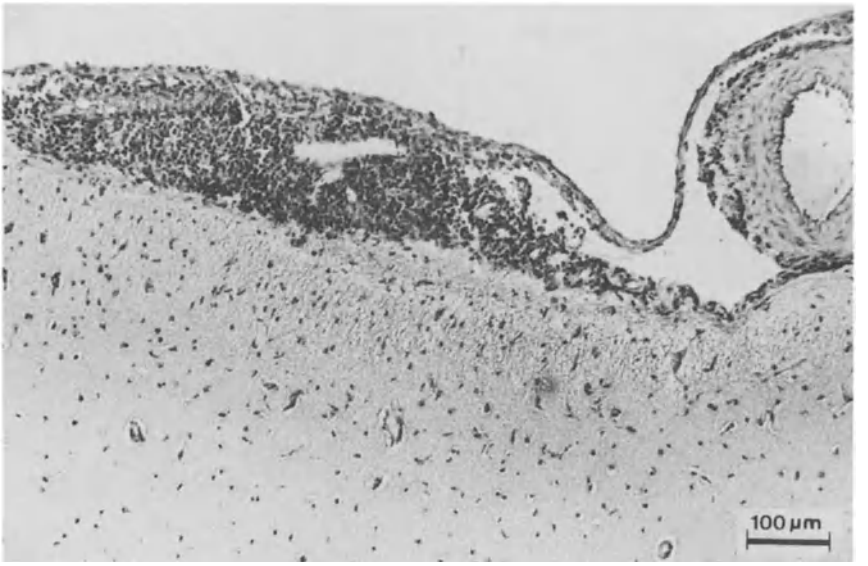

Fig.7. Heavy inflammation of the leptomeninges over the pyriform lobe.H.E.

median fissure, the posterior median sulcus (cf. Fig.9) and around nerve roots, especially the posterior ones. The meningitis is an early feature of the pathological lesions and has been detected 1-2 weeks after infection (17,48, 50,52). It is sometimes very marked, especially shortly after infection. The intensity of the inflammation is variable and does usually wane with time, although it may still be present 6 to 7 years after infection (57). The inflammatory infiltrates consist of mononuclear cells, lymphocytes are usually most prominent, followed by macrophages with some, usually discrete admixture of plasma cells.

Concomitantly with the evolution of the meningitis a pleocytosis of the cerebrospinal fluid (CSF) is observed (50,52,56,58,59). The number of cells usually reaches maximal levels approximately one month after infection and wanes within a period of a few months to remain at slightly increased levels for some period of time (52,58,59). Occasionally a later rise, several months after infection, is found and irregular fluctuations have been observed in

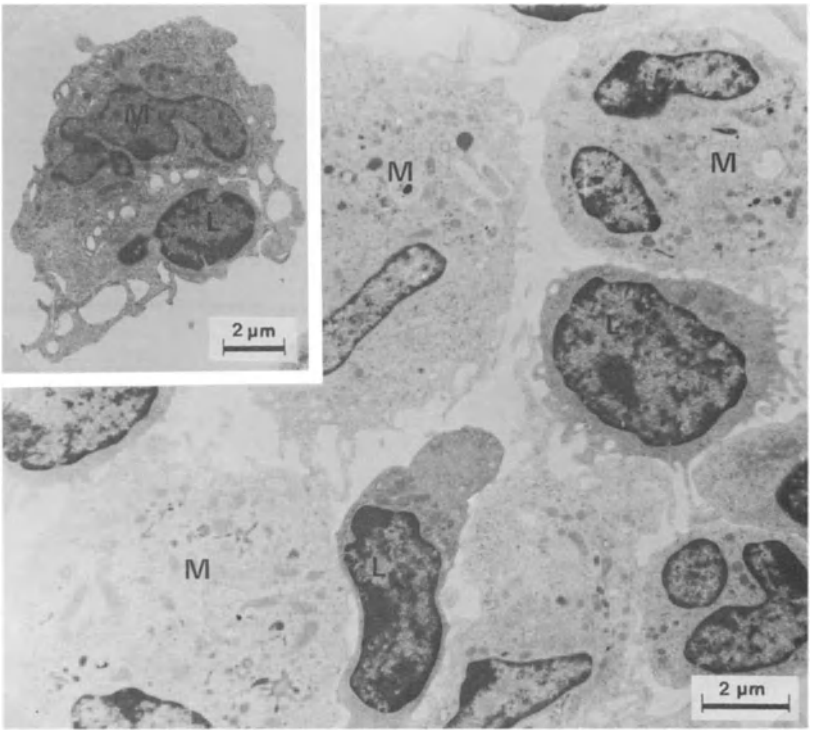

Fig.8. Cellular exudate in the CSF consisting of macrophages(M) and lymphocytes(L). Inset: Macrophage in close contact with a lymphocyte.

long-term studies (58) with peaks of pleocytosis up to approximately 1000 cells per µl occurring as late as 7 or 8 years after infection (G.Pétursson et al., unpublished results). As there is a correlation between the number of cells in the CSF and the severity of pathological lesions (58) the fluctuation in the pleocytosis indicates a remitting lesion activity in the CNS.

A cytological analysis of the cellular exudate in the CSF shows that it is of a similar composition as the inflammatory infiltrates in the meninges (Fig. 8). The only difference being in the relative proportion of the cell types. Thus we found, with an occasional exception, macrophages to be more numerous than lymphocytes in the CSF and

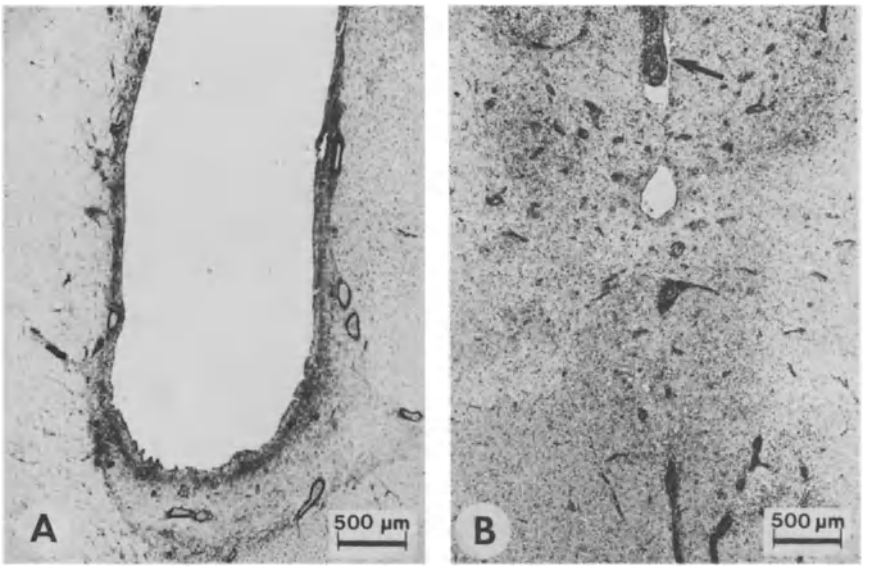

Fig.9. A) Confluent periventricular inflammation around the lateral ventricle. Perivascular infiltrates extend into adjacent neuroparenchyme.H.E. B) Inflammation of the spinal cord radiating from the central canal. Pronounced meningitis in the anterior median fissure (arrow). H.E.

plasma cells were exceedingly rare (56,58). This may possibly be a reflection of differences in migratory potential of these cells. Griffin et al. (59) found lymphocytes to be more numerous than macrophages, but they used a different method to enrich for CSF cells.

Another finding of interest observed in an ultrastructural analysis was the observation of myelin fragments in the CSF sediment (56) possibly indicating an active myelin breakdown. Tests for myelin basic protein in the CSF have revealed a transient elevation (59). The escape of myelin proteins into the CSF may have implications for the pathogenesis of lesions. Similar findings have been reported in human demyelinating diseases (60,61) and it has been suggested that myelin entering the CSF may lead to auto-sensitization to myelin proteins (62). Another possible indication of myelin breakdown was the observation of lipid droplets in a substantial proportion of the

macrophages. These lipophages were somewhat more numerous late in the course of the infection (56).

The cellular exudate does not exclusively represent a

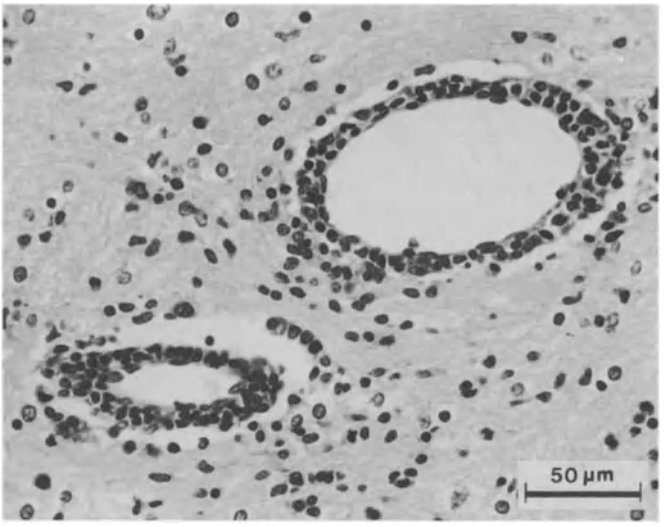

Fig.10. Perivascular infiltrates in the white matter of the cerebrum.H.E.

spillover from the inflammatory infiltrates of the meninges, because concomitantly inflammation evolves in the brain, where the main localization according to a semi-quantitative analysis (24,55) is subependymal, and furthermore an inflammatory infiltration of the choroid plexus is an early and common change observed. According to our observations initial changes were found in half of all cases sacrificed and examined 2 weeks after infection (52) and consisted of subependymal inflammation frequently starting as small perivascular sleeves of inflammation scattered underneath the ependymal lining of the ventricles but in more severe cases becoming more diffuse and confluent (Fig.9) sometimes bordering the entire ventricular system of the brain and frequently extending into the spinal cord along the central canal (Fig.9). Sometimes the ependymal lining sloughs of the surface. From the periventricular localization the inflammation extends, first as perivascular infiltrates into the adjacent neuroparenchyme (Fig.10), especially the white

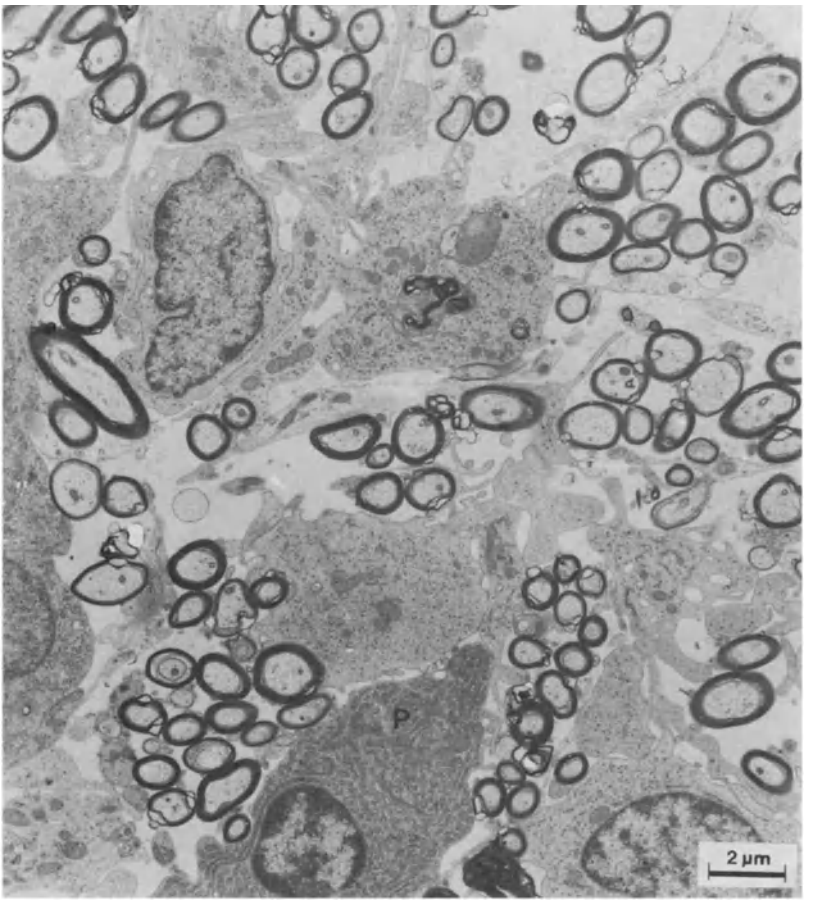

Fig.11. Infiltration of inflammatory cells into the white matter, pushing well-preserved myelinated fibres apart. P=plasma cell.

matter although not sparing the adjacent nuclei. At first the perivascular infiltrates are confined to the Virchow-Robin space, leaving the limiting lamina intact. With progression of the lesions they become confluent and may in extreme cases involve almost the entire white matter. The cerebral cortex is rarely affected. An occasional glial nodule or discrete perivascular inflammatory cuffs have been observed in the cortex, usually in conjunction with

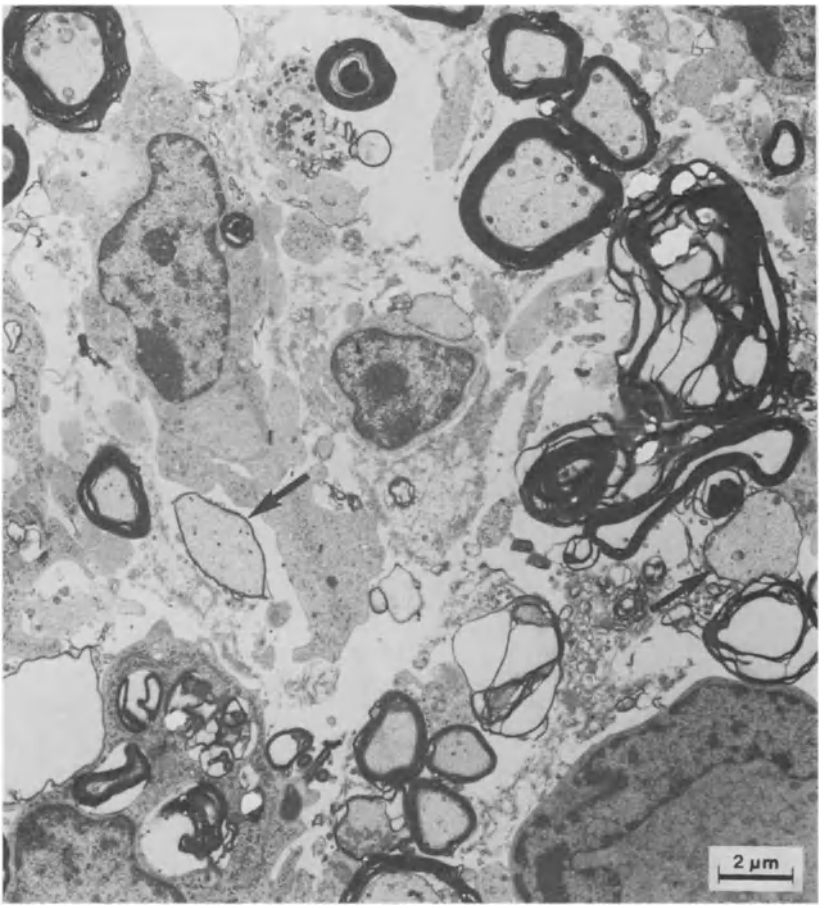

Fig.12. Dissolution of white matter with inflammatory cells, secondary myelin breakdown and primary demyelination (arrows). Several intact myelinated fibres.

meningitis or inflammation of the subcortical white matter. Glial nodules are also sometimes present in the white matter.

In the spinal cord inflammation extends in a similar manner from the central canal into the adjacent grey matter but may occasionally evolve in the white columns without apparent relation to an inflammation around the central canal.

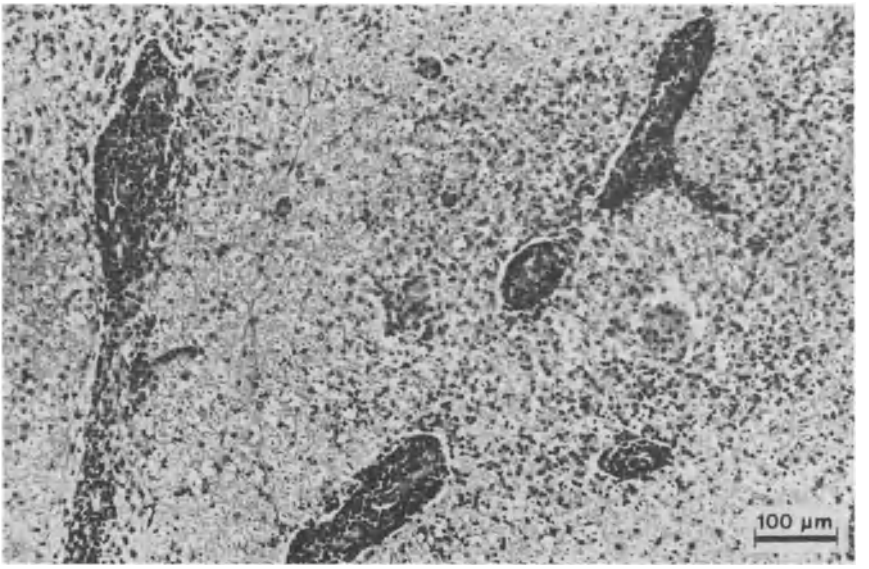

Fig.13. Confluent inflammation of lateral column of the spinal cord with partly necrotic white matter.H.E.

Initially the myelin is spared, the inflammatory infiltrates push the myelinated fibres apart (Fig.11), which are sometimes surprisingly well preserved, even in areas with almost complete dissolution of the neuroparenchyme (Fig.12). Eventually the myelin is broken down. Sometimes multiple small foci of myelin breakdown are observed but usually extensive foci of liquefaction necrosis with destruction of myelin and axons (Fig.13), i.e. secondary demyelination, are found, with massive infiltration of macrophages filled with phagocytosed material ("gitter cells"). Besides liquefaction necrosis occasional foci of coagulative necrosis have been observed with palisading of cells around the margins (Fig.14). This is obviously not an effect of the inoculation since such foci were present distant from the site of inoculation and were sometimes observed many years after infection. Furthermore analogous lesions have been observed in natural infection (13). Besides a secondary myelin breakdown a rather sharply demarcated foci of primary demyelination have been observed in long-term studies (57).

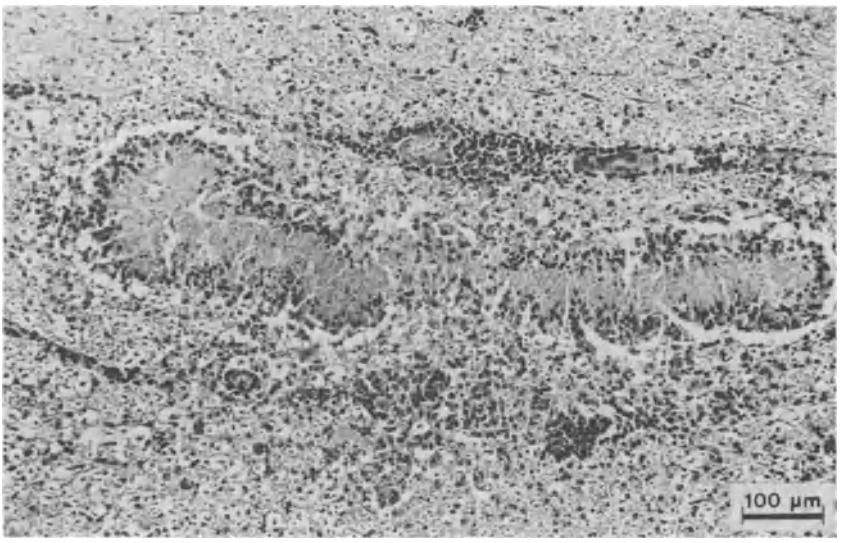

Fig.14. Coagulation necrosis in the white matter.H.E.

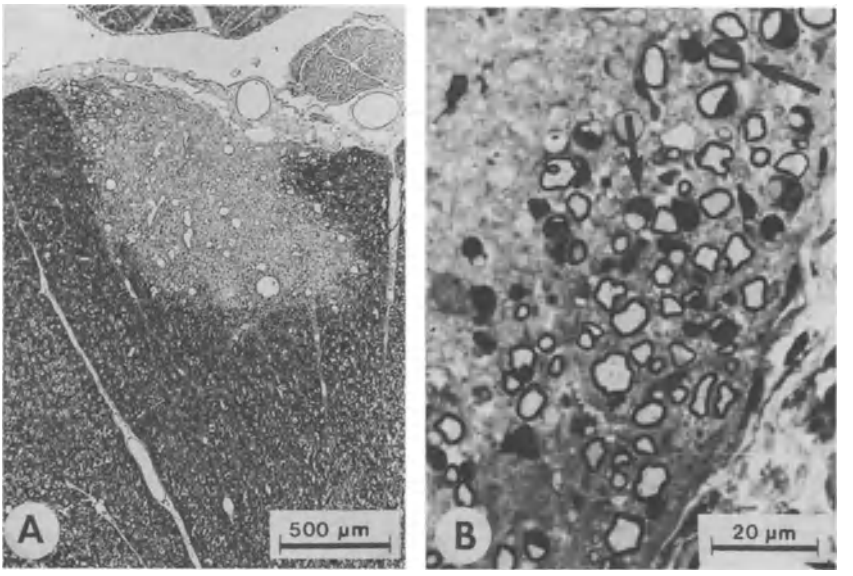

Fig.15. A) Sharply demarcated plaque of primary demyelination in the spinal cord. Klüver-Barrera. B) Remyelination partly by Schwann cells(arrows).Toluidine blue.

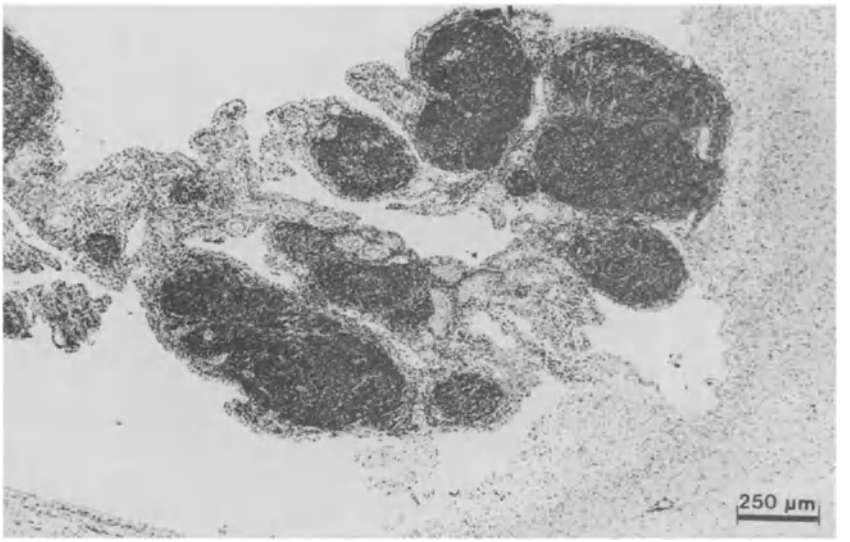

Fig.16. Inflammation of the choroid plexus of the lateral ventricle with numerous lymph follicles.H.E.

These foci have been observed in sheep coming down with clinical signs from 2 to 7 years after infection, especially in the spinal cord (Fig.15). These foci are highly reminiscent of chronic active or chronic silent plaques found in multiple sclerosis (M.S.) and may show signs of remyelination frequently with a peripheral type of myelin (Fig.15).As already mentioned an inflammation of the choroid plexus in the lateral, third and fourth ventricles is an early and common feature of the pathological lesions observed in visna. It differs in degree, from discrete infiltration of lymphocytes, some macrophages with a varying admixture of plasma cells to very pronounced lymphoid proliferation with formation of lymph follicles (Fig.16) frequently with active germinal centers where mitotic figures are also observed. Mitotic figures are also to be seen in the inflammatory infiltrates in the neuroparenchyme.

Neurons are spared except in areas with frank necrosis and are often well preserved where there is a pronounced

inflammation. This is in accord with results of in situ hybridization (63) and immunohistochemical studies of the brain (64). Neither the viral genome nor expression of viral proteins have been detected in neurons by these methods whereas astrocytes and oligodendrocytes do apparently harbour the viral genome (63).

A proliferation of astrocytes has been observed around perivascular infiltrates and bordering necrotic foci, but no indication of a diffuse response has been observed. Except for some swelling of endothelial cells of capillaries and venules in inflammatory foci, the vessels rarely show any changes. Occasionally slight intimal thickening in arteries and veins is observed and some perivascular fibrosis has been found in an occasional case.

Multinucleated giant cells, the hallmark of the cytopathic effect of infection with the MVV in tissue culture, are apparently very rare, thus we have not found any in the brain lesions in our series using the K1514 virus strain. This is probably a reflection of the low virus titres observed in the CNS (55). In experiments done with a highly neurovirulent strain, that we selected for by serial passage of virus through sheep (65), multinucleated giant cells were occasionally found (G.Georgsson et al., unpublished results).

We have not found any changes in the peripheral nervous system. Thus nerve roots, the sciatic, optic nerves and retina were normal in our series. But Sigurðsson et al. (48) occasionally found an extension of the inflammation of the meninges into adjacent spinal ganglia and nerve roots as well as isolated inflammatory foci in peripheral nerves at some distance from the nerve roots. Our observations indicate that affection of the peripheral nervous system must be exceedingly rare.

We have not observed a change in the character of the pathological lesions with time. Thus the composition of the inflammatory infiltrates was similar in sheep sacrificed 10 years after infection to those observed 2 weeks after

infection, i.e. lymphocytes were in general most conspicuous, with considerable infiltration of macrophages and a variable number of plasma cells. And other features of the pathological lesions were found at different times after infection, except plaques of primary demyelination, which were apparently a rather late manifestation. Thus in contrast to findings in American sheep (50,66) categorization of pathological changes in our material into acute and chronic is not warranted. This difference is probably a reflection of different susceptibility of the Icelandic versus the American breeds, which is consistent with the fact that in general a 10-fold greater concentration was needed to induce encephalitis in American breeds than in Icelandic sheep (50,66,67,68). The only chronic lesions observed in our material were glial scars at the site of inoculation, which was occasionally included in the planes of sections taken for histological examination, and in general an ongoing inflammation was found in other areas often at distant sites from the scarring. This scarring obviously represents reparative processes occurring where there is a destruction of tissue. There is no question that if sheep survive long enough after liquefaction necrosis has occurred glial scars or cystic transformation of the nervous tissue does occur after the necrotic tissue has been absorbed (48).

The results of a long-term study, where a group of 20 sheep were infected simultaneously and followed until appearance of clinical signs, the period of observation being as long as 11 years, indicate that lesion activity may be remitting, and inflammatory lesions, which are not accompanied by tissue breakdown, may resolve and reappear later. This interpretation is supported by the fluctuation in the number of cells in the CSF, that may repeatedly show peaks at different intervals and the fact that lesions of comparable character and severity are observed from one month to many years after infection (52,57).

Mastitis

The lung- and CNS- lesions caused by infection with the MVV have been known for 75 and 35 years respectively. More recently there is increasing evidence, that the mammary gland is also a target of infection with MVV. De Boer (69,70) isolated MVV from milk of infected ewes in the Netherlands indicating that infection might spread from ewes to suckling lambs and a few years later the occurrence of mastitis was reported in the United States (11) and Germany (12). Subsequently there have been several reports on mastitis in natural infection (13,14,16,71) and transmission experiments (15,16). Both field studies (14,16,71,72) and transmission experiments (15,16) indicate that the mammary gland may be more susceptible to infection with MVV than the traditionally well known targets, i.e. lungs and CNS. Mastitis was not reported during the epizootic in Iceland. It may have been overlooked because the clinical symptoms of the lung and CNS effects were more alarming. An ill-thrift of lambs of maedi-visna affected ewes was, however, frequently observed (P.A.Pálsson, personal communication) possibly due to lower milk yields due to an effect on the mammary gland.

Macroscopic changes. There is a diffuse induration of both udder halfs (14,71), which differs from mastitis caused by bacterial infection, where according to a comparative study by van der Molen et al. (14) macroscopic changes are nodular and usually limited to one udder half. Little milk can be expressed from the teats, which according to a study of Anderson et al. (71) on some ewes shortly after parturition can be caused by compression of the lactiferous sinuses, since he found a profuse flow of milk when a biopsy was made caudo-dorsal to the teat. Van der Molen et al. (14) in their study of ewes in mid-lactation found evidence of decreased milk secretion and occasionally only a sparse watery secretion could be expressed from the cut surface of affected glands, which had lost their normal granularity and appeared smooth and homogenous. This may simply reflect

different stages in the development of the mastitis. The mammary lymph nodes are enlarged (14).

Microscopic changes. The common and most prominent histologic feature described both in lactating and

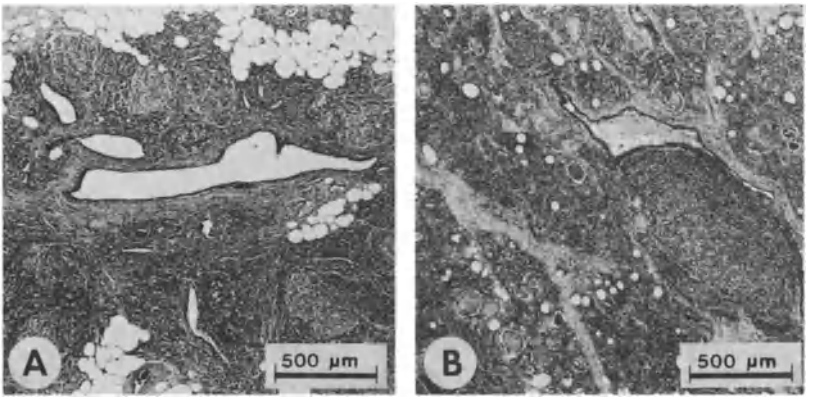

Fig.17. A) Inflammation in a non-lactating gland with pronounced lymphoid hyperplasia around a duct.H.E. B) Inflammation in a lactating gland with lymph follicle bulging into a duct and diffuse inflammatory infiltrates and some fibrosis with destruction of mammary lobes. Remnants of secretion are present.H.E.

non-lactating mammary glands is a lymphoid proliferation with formation of lymphatic nodules (11,12,13,14,15,16,71) sometimes with active germinal centers. These nodules are both found periductally, where they may protrude into the lumen and cause compression and distortion of the ducts, but are also found in the lobules (Fig.17). Besides the lymphatic nodules there is a diffuse infiltration of lymphocytes in the interstitium and the lobules with a number of activated lymphoblasts (16). Besides lymphocytes Olivier et al. (13) found some admixture of plasma cells which are common according to the ultrastructural study by Deng el al. (16). They also found an occasional eosinophil and mast cells whereas neutrophils are not found in the inflammatory infiltrates. Apparently macrophages are rare and have not been reported as a feature of the inflammatory response except possibly by Griem and Weinhold (12) who used the term

lympho-histiocytic for the inflammatory infiltrates observed in their study. An infiltration of lymphocytes is found into the ductal and the acinar epithelium and in the lumens of these structures. The ductal epithelium can be hyperplastic (15) but frequently shows signs of degeneration. It is often necrotic and sloughs into the duct lumens. In lactating glands there is a diffuse infiltration of the lobules with destruction of the acini, which may contain remnants of secretion, and a varying degree of fibrosis (Fig.17) is present (14,71). Virions were not detected by electron microscopy (16) and multinucleated giant cells have not been observed, probably a reflection of low virus titers.

Arthritis

A chronic arthritis associated with infection with the American strain of MVV, PPV, has been reported in the United States. Oliver et al. (13) found a bilateral chronic carpal arthritis in a group of sheep with natural PPV infection, isolated virus from the affected joints and succeeded in inducing arthritis by intraarticular infection (17). In a later study Cutlip et al. (18) found arthritis in almost 50 per cent of a group of sheep with experimental (by the intravenous and intrapulmonary route) and natural infection, most commonly affecting the carpal joints and more rarely the tarsal joints.

A spontaneuosly occurring arthritis due to infection with MVV has not been reported in other countries. This can possibly be explained by variation in virus strains. Thus the PPV shows a greater nucleotide homology with the caprine arthritis-encephalitis virus (CAEV), where the main clinical manifestation in adult goats is arthritis (73, see also chapter by Cork), than the Icelandic strain of MVV (74). MVV does, however, grow in synovial cells (75) and Larsen et al. (76) were able to induce synovitis in both Icelandic and Norwegian sheep by intraarticular inoculation of two different Norwegian isolates of MVV, which indicates

that a subclinical arthritis may be expected to occur in infection with other strains of MVV.

Macroscopic changes. The initial changes according to experimental infection is a subtle swelling of the joints, which feel hot (17). The synovial fluid is increased, more

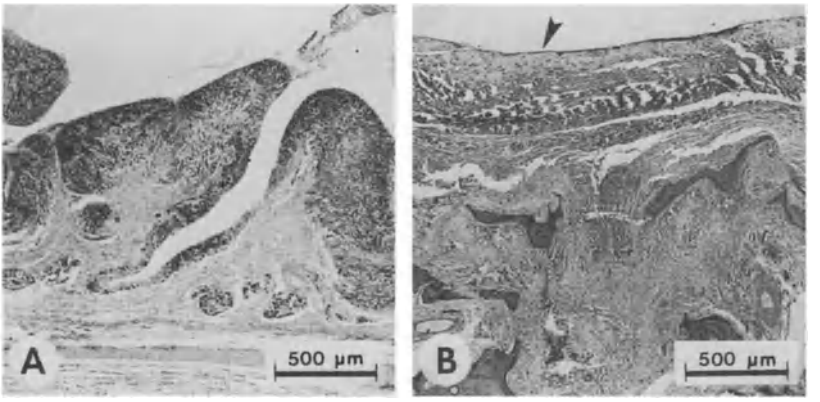

Fig.18. A) Villous synovitis with heavy inflammation. H.E. B) Articular cartilage replaced by fibrotic tissue (arrowhead). Destruction of subchondral bone with fibrosis. H.E.

viscous and cloudy (17,73). There is a distinct increase in leukocytes, almost exclusively lymphocytes and monocytes (17). The synovium is hyperplastic with prominent villi. Adjacent tendon sheaths are affected and in early phases hyperaemia and petechial haemorrhages are observed (17). With progression of the lesions a distinct firm swelling of the joints is noted, mainly caused by fibrous thickening of the capsule and periarticular tissues, whereas the exudate into the joint cavity does subside (13,18). The articular cartilage may show extensive erosions (13,18) and chalky debris from eroded cartilage is found in the joint cavity (18). Furthermore fractures of the articular surfaces and subchondral bone do occur (18).

Microscopic changes. Initially a chronic villous synovitis with oedema, hyperaemia and varying degree of inflammatory infiltrates with lymphocytes and plasma cells,

frequently in form of conspicuous perivascular cuffing is observed (Fig.18). Often formation of lymph follicles with active germinal centers are found in the synovia (17,18,73) as well as fibrin deposits on or in the synovial villi (17). A hyperplasia of the synovial cells is observed (73). The inflammation extends to periarticular structures, where extensive fibrosis and necrotic areas with calcification are noted (13,18). Foreign body granulomas in the synovial membrane as a response to necrotic cartilage and bone fragments have been described by Cutlip et al. (18) who also found media necrosis and inflammation of small arteries and arterioles in the periarticular tissues. There is an erosion of articular cartilage that may be replaced by fibrous tissue and a destruction of the subchondral bone (Fig.18), which may eventually lead to fibrous ankylosis of the joints (13,18). Osteophytic outgrowths have been observed (13). Oliver et al. (13) found lesions in the nuchal ligament in one of their cases, similar to those observed in the periarticular structures. In addition to virus isolation from affected joints (17), Larsen et al. (76) could demonstrate anti-viral antibodies in the synovial fluid in their experimental infection.

The organ spectrum of infection with the MVV in sheep, i.e. encephalitis, pneumonia, mastitis and arthritis, as well as the main features of the pathological lesions, is similar to that observed in infection with a related lentivirus (CAEV) in goats (see chapter by Cork). Furthermore human immunodeficiency virus (HIV), a lentivirus related to MVV (77), does in part have a similar tissue tropism, i.e. it infects lungs and CNS (78,79,80,81). A comparison of the pathological changes observed in these organs in AIDS with those found in maedi and visna is problematic, as it is difficult to dissect out which lesions observed in AIDS are primarily due to the virus and which are caused by opportunistic infections or therapeutic measures. The lymphoid interstitial pneumonia primarily found in pediatric AIDS cases (81) certainly resembles maedi.

PATHOGENESIS

Viraemia is found both in natural and experimental infection using various routes of inoculation. The viraemia develops within a couple of weeks after either intracerebral or intrapulmonary inoculation of MVV (10,52) and plays a decisive role in the spread of the infection to various organs, resulting in a systemic disease.

In the blood the infection seems to be mainly cell-associated, thus we have never found free virus in plasma (52). A very low proportion of leukocytes of the peripheral blood carries the virus or in the order of 1 in 100,000 or 1,000,000 (52). According to Narayan et al. (82) the main target cells in the blood are monocytes. There is, however, a restriction of viral replication in the circulating monocytes but on maturation into macrophages *in vitro* replication of the virus and expression of viral antigens is turned on at a low level (83), similar to that observed in selected populations of tissue macrophages (84). In contrast to these findings De Boer (70) found lymphocytes of peripheral blood to be infected, which is in accordance with some of our findings on peripheral blood (52) and lymph (85). By immunohistochemical staining (64) on sections from the CNS lymphocytes and plasma cells were in addition to macrophages found to express viral antigens. This apparent discrepancy may be due to difference in tissue tropism of the virus in different breeds of sheep. The variation in the incidence of different organ manifestations in different breeds of sheep would support such an explanation. In this context it is of interest, that we have by immunohistochemistry (64) and *in situ* hybridization (63) in Icelandic sheep, which seem to exceptionally susceptible to CNS disease, found that a wide variety of cells in the CNS are permissive for infection with MVV, such as oligodendrocytes, astrocytes, endothelial cells, pericytes and fibroblasts.

Narayan et al. (86) have demonstrated a novel type of interferon induced in lymphocytes interacting with infected macrophages that may lead to amplification of the response of lymphoid tissue, partly through induction of Ia antigens on macrophages (87), but lymphoid proliferation is a common denominator of pathological lesions in the various organs affected with MVV, i.e. lungs, mammary gland, joints and CNS (see above).

The evolution of pathological lesions in organs with a rich blood and lymphatic circulation, where there is a constant migration through the tissues of leukocytes from one system to the other can be easily explained. It is, however, more difficult to explain how MVV — infected leukocytes get into the CNS to induce lesions, as the CNS is commonly regarded as an immunological privileged site. We have in analysing the blood-brain barrier not found evidence that it is disrupted (58), at least not permanently, although a temporary disruption can not be excluded. It is very likely, however, that a breakdown of the blood-brain barrier is not a necessary condition for leukocytes to enter the CNS, as there is evidence that there is some lymphocytic traffic in the normal CNS (88). Furthermore endothelial cells of the brain, that express viral antigens (64) might contribute to homing of sensitized lymphocytes into the CNS.

According to our results the development of early lesions in the CNS is immune-mediated. Thus in experiments where a group of sheep were immunosuppressed and challenged by intracerebral inoculation of MVV, at the height of suppression only 1 out of 8 suppressed sheep developed lesions in comparison with all 8 that were challenged but not immunosuppressed (89). Furthermore by boosting the immune response at a time when infection and lesion development had been established in the brain more severe lesions were induced than in infected but not immunostimulated sheep (90). The damaging immune response is apparently directed against virus-induced antigens, as the severity of lesions is dose-dependent (85) and shows a correlation with the

frequency of virus isolations (52). In short-term experiments there was no indication of an autoimmune reaction against myelin antigens (91). In the related lentiviral infection of goats results of immunosuppression (92) and immunostimulation (93) indicate a comparable pathogenetic mechanism in lesion development.

It is not known which limb of the immune response, i.e. humoral or cell-mediated, is imperative for the development of lesions although according to the time scale of the immune response it seems more likely that the cell-mediated immune response is primarily involved as this effect of immunosuppression is observed when cell-mediated immune response to the infection has developed (94), and before neutralizing antibodies can be detected. The effect of immunosuppression has only been tested in relatively short-term experiments, lasting only 5 weeks, as it was not feasible to apply immunosuppression for a longer period because of supervening opportunistic infections.

The pathogenetic mechanism may, however, change with time. The different character of the late lesions of primary demyelination might be interpreted as an indication of such a change. The recent finding, that oligodendrocytes may harbour the virus genome (63), would support the idea, that demyelination could be caused by immune-mediated destruction of oligodendrocytes. However, expression of viral antigens in oligodendrocytes could not be detected by immunohistochemistry (65). The demyelination could be due to direct cytopathic effect on oligodendrocytes as observed in progressive multifocal leukoencephalopathy of humans infected with papovavirus (95) or possibly due to non-specific destruction by proteases and lymphokines released by inflammatory cells, a so-called "bystander" demyelination (96).

As already mentioned several lines of evidence indicate that lesion activity may be remitting, usually subclinical although occasionally a remitting clinical course is observed. The alternating peaks of lesion activity may be

due to intermittent release of the restriction of virus replication found in the infected host. Another possible explanation would be a fluctuation in the damaging immune response. In this respect it is of interest that in long-term experiments the cell-mediated immune response shows irregular fluctuations (97,98).

It has been suggested that the emergence of antigenic variants which would escape the immune response of the host could play a role in development of lesions (99,100,101). This is an interesting hypothesis since antigenic variants are commonly observed both in vivo. However, our analysis of antigenic variants with neutralizing antibodies in a group of 20 long-term infected sheep for 7 years (102) as well as those of Thormar et al. (103) did not show any increase in variants with time after infection and they did not replace the infecting strain but coexisted with it. Furthermore we did not observe any correlation between the emergence of variants with either evolution of pathological lesions or clinical signs. However, other genomic changes of the virus that are not detected by neutralizing antibodies (104) may nevertheless play a role in the pathogenesis and the development of lesions, for example by a possible effect on the expression of the virus genome. But these problems have not been resolved nor have many other aspects of the pathogenetic mechanisms at later stages in this persistent infection.

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4

MAEDI-VISNA. ETIOLOGY AND IMMUNE RESPONSE.

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ABSTRACT

The causative agents of maedi, a slow progressive pneumonia and visna, a slow progressive demyelinating encephalitis of sheep are classified as lentiviruses, a subgroup of retroviruses. The lentivirus subgroup also includes caprine arthritis-encephalitis virus (CAEV), equine infectious anemia virus and human immunodeficiency virus (HIV) which causes AIDS. These viruses are not oncogenic but cause cytolytic changes when replicating in permissive tissue culture cells. In the host they cause a persistent infection, characterized by a severe restriction of viral replication with a small number of cells infected, many of which carry the viral genetic information in the form of provirus DNA. In spite of a fairly good antibody response the host never gets rid of the virus, which results in a life-long infection.

INTRODUCTION

The epidemiological behaviour of maedi in two separate areas of Iceland following the importation of Karakul sheep in Iceland clearly indicated that the disease was contagious and that its spread was facilitated by close contact and crowding of sheep (1, see also chapter by Pálsson). Experimental work showed that the disease could be transmitted to healthy animals by contact with diseased sheep and intrapulmonary inoculations of lung material (2). Later a virus was isolated from maedi lungs (3) and shown to be able to produce the disease in healthy lambs (4). When

visna was observed in one of the two regions of Iceland affected with maedi it was at first confused with scrapie which had been known in Iceland since early in this century (5). Soon it became clear that visna was different from scrapie and it was shown to be transmissible by intracerebral inoculation of brain material from field cases of visna into healthy animals (6,7). These experiments pointed to a viral etiology which was proven by isolation of the visna virus in tissue culture and subsequent reproduction of the disease by intracerebral inoculation of cultured virus into healthy lambs (8). Visna virus was thus the first lentivirus to be isolated and characterized. After both the viruses of visna and maedi had been grown in tissue culture comparative studies showed the two agents to be closely similar or practically identical and it became clear that visna and maedi are two clinical and pathological manifestations of infection with the same agent (4,9,10,11). These results were confirmed by virus isolation in Holland from zwoegerziekte, a sheep pneumonia resembling maedi (12) and since then similar viruses have been isolated from many countries of the world (1, see also chapter by Houwers).

STRUCTURE OF THE VIRUS

Ultrastructure

Maedi-visna virus (MVV) belongs to the lentivirus subfamily of retroviruses, the other two subfamilies are oncoviruses and spumaviruses (13). Other viruses of the lentivirus subfamily include caprine arthritis encephalitis virus (CAEV) a close relative of MVV, the more distantly related equine infectious anemia virus and the human immunodeficiency virus (HIV) (14).

Under the electron microscope the virions of the lentivirus subfamily look alike but differ slightly from other retroviruses. Thus the electron-dense line of budding virus particles is closer to the cytoplasmic membrane than in other retroviruses. Furthermore the core or the nucleoid

of lentiviruses seems to be elongated or rod-like rather than round (15).

Maedi-visna virions are formed by budding from the cytoplasmic membrane of infected cells (Fig. 1A). Crescent-shaped budding structures form a particle with a relatively electron-lucent center. This structure appears to condense and to form a smaller (ca. 100 nm) particle with a central dense core or nucleoid about 40 nm in diameter (16,17,18). Such mature virions are found outside infected cells (Fig. 1B). With good resolution a thin internal membrane can be discerned between the central dense nucleoid and the outer membrane (19). By negative staining the virus can be seen to be covered with knobs or spikes about 9 nm in length

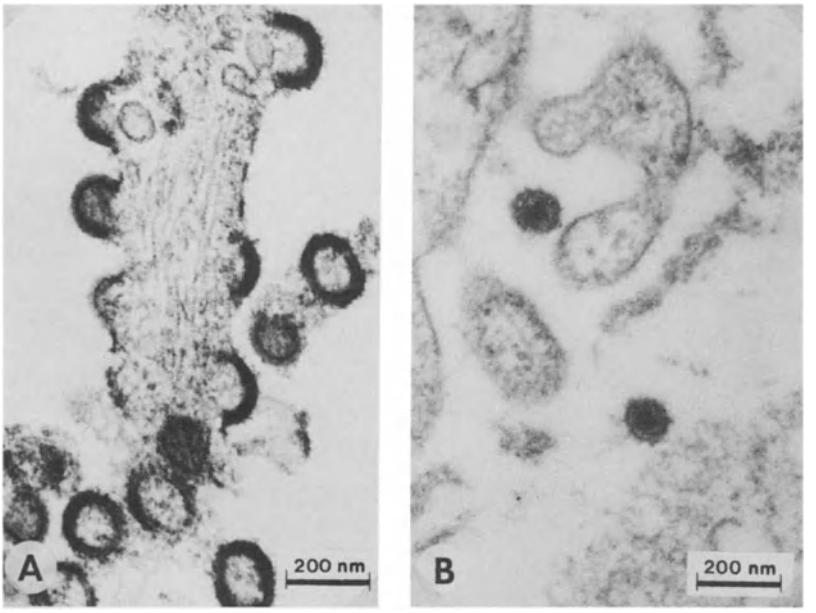

Fig. 1. A) Electron micrograph of MVV budding from the surface of a sheep choroid plexus cell in culture.
B) Complete extracellular MVV particles.

(20,21). Inside the cytoplasm of infected cells multi-layered structures resembling budding crescents can sometimes be seen, apparently incomplete or abortive forms of virions (22).

Physicochemical properties

MVV is readily inactivated by ethyl ether, chloroform, formaldehyde, ethanol and phenol as well as by trypsin treatment. It is stable at -50°C for months and for years at lower temperatures. The titer is not severely affected by several cycles of freezing and thawing. In medium containing 1% sheep serum 90% of the infectivity is lost after 4 months at 4°C, 9 days at 20°C, 24-30 hours at 37°C and 10-15 minutes at 50°C. It is rather stable at pH values between 5.1 and 10. The relative resistance to UV-irradiation is similar to that of oncoviruses (10). In sucrose the isopycnic density is 1.15-1.16 g/ml. As with other retroviruses the sedimentation coefficient is about 600S and the isoelectric point of the virus is 3.8. The probable overall composition of the virus is 60% protein, 35% lipid, 3% carbohydrate and 2% RNA (23).

Virion nucleic acid

The virus genome consists of a single-stranded 60-70S RNA molecule with an extensive secondary structure. On denaturation two or three 35S subunits are released. The virion also contains ribosomal and low molecular weight RNAs of cellular origin (24,25,26,27). The RNA genome has a binding site for $tRNA^{Lys}$ which serves as a primer for DNA synthesis (28). The 35S subunits have a molecular weight of 3.6×10^6, are of plus strand polarity and have a poly A tract at the 3' end (29,30). The subunits seem to contain essentially the same genetic information i.e. the genome is polyploid (probably diploid) (31). The nucleotide sequence of the visna virus genome has now been determined from cloned proviral DNA and contains 9202 nucleotides (28). The overall base composition is: U=21.4%, C=15.4%, G=26.1%,

A=37.1%. The organization of the virus genome is described in the chapter by Pyper et al.

Virus proteins

The number of polypeptides of mature virions have been variously reported as 10 to 25 (32,33,34). Of those only 4 polypeptides comprise about 80% of the protein mass of the virion i.e. p25, p16, p14 and gp135. The first three are the so-called gag proteins and p25 is the main component of the virus core and comprises about 40% of the protein mass of the virion. The p16 polypeptide corresponds to the N-terminal part of the gag precursor and the p14 is apparently a nucleic acid binding protein with an actual molecular weight about 8800, but since it is a highly basic protein it migrates so slowly on SDS-PAGE gels that its apparent molecular weight is 14000 (28). The surface glycoprotein gp135 is thought to be the main constituent of the surface spikes of the virion. Antibodies raised against gp135 have been shown to have virus-neutralizing activity (35). In gel immunodiffusion sera from infected animals commonly show two different precipitation lines, one corresponds to the gp135 and the other to p25 (36). Sometimes sera from infected animals only show one line which corresponds to the gp135 envelope protein.

An RNA-directed DNA polymerase or reverse transcriptase is a typical virion component of retroviruses, and coded for by the pol gene of the virus (37,38,39). Purified visna virus has been reported to contain two forms of such an enzyme, one with a sedimentation coefficient of 6.9S and an apparent molecular weight of 135,000 and another 6.3S glycoprotein (40). The first form was reported to be a dimer of two molecules of 68,000 molecular weight. The 6.3S form has an apparent molecular weight of 118,000 and may be of cellular origin. The coding capacity of the pol gene in visna corresponds to a protein of 126,500 m.w. including an endonuclease-integrase domain and sequences corresponding to a viral protease (28). The visna virus reverse trans-

criptase shows a preference for Mg++ over Mn++ for optimal activity as some other retroviral reverse transcriptases.

MVV has been reported to contain neuraminic acid in its envelope (41). Although the MVV itself has not been found to agglutinate red cells of various species, it will inhibit haemagglutination by influenza viruses and this haemagglutination inhibition activity can be abolished by treatment with neuraminidase. Such treatment did not influence virus attachment to sheep cells nor the cell-fusing activity of MVV.

REPLICATION OF MVV

Replication in tissue culture

MVV is cytopathic in permissive tissue culture cells (Fig. 2) and differs in this respect from many retroviruses. The cytopathic effect (CPE) consists of the formation of rounded cells of increased refractibility with numerous processes (star-shaped or spider-like cells) and the formation of multinucleated syncytia (8). This leads to a destruction of the cell monolayer in one or two weeks, depending on the virus input, and to high titers of virus progeny in the culture fluid (10^7-10^8 TCD_{50} per ml.) or 50-100 PFU per cell (42). High concentrations of input virus may cause direct fusion of cells within 30-60 minutes. This cell-fusion from without does not depend on virus replication and can be produced by UV-inactivated virus. Cells that do not support virus replication such as hamster cell lines may be susceptible to this direct virus-induced fusion (43).

Adsorption of virus to cells is completed in about 2 hours at 37°C. There is a period of latency of 20-24 hours followed by an exponential increase of virus for another 15 hours or so. Then there is a more gradual increase in virus titer until a maximum is reached about 50 hours after infection. The first signs of CPE are detected between 24 and 36 hours after infection with a high input multiplicity of virus (20 TCD_{50} per cell) (42).

MVV enters the cell by fusion. Reverse transcription of the viral RNA seems to be initiated in the cytoplasm and completed in the nucleus (44,45,46). The completed viral DNA is mostly in a linear duplex form with a gap in the plus strand but there is also a small fraction of DNA in a circular form (47,48,49). Some of the viral DNA is associated with high molecular weight cellular DNA but covalent linkage of viral and cellular DNA has not as yet been established in the usual cell-virus systems, but may

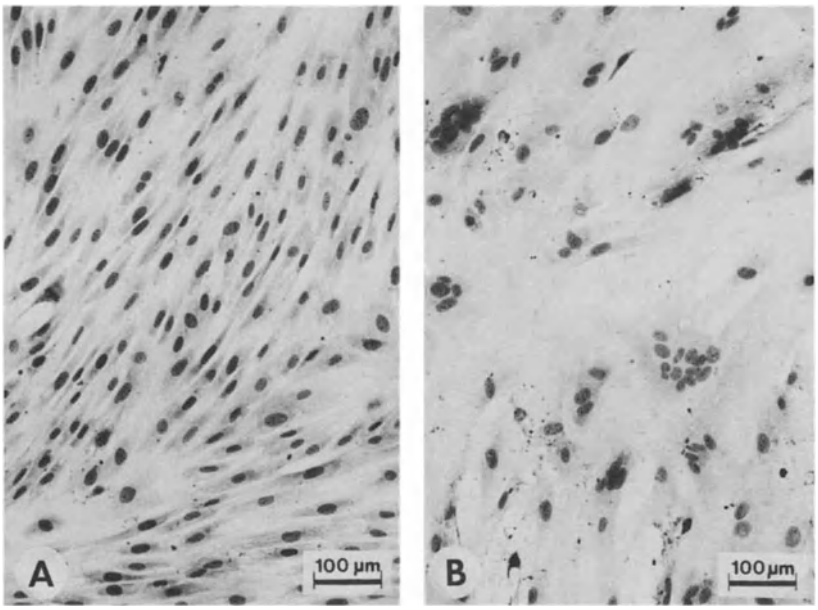

Fig. 2. A) Uninfected sheep choroid plexus cell monolayer in culture with elongated fibroblast-like cells. H.E. stain. B) Cytopathic changes in a MVV-infected choroid plexus culture. Multinucleated syncytia and spider-like cells. H.E. stain.

occur in fetal cornea cells that divide more rapidly than choroid plexus cells (50). Viral DNA synthesis continues through the replication cycle and reaches several hundred copies per cell (14). The initial amplification of viral

genomic information by synthesis of viral DNA is necessary for full yield of viral RNA and infectious progeny virus (49,51). Viral RNA synthesis starts in the nucleus of the cell about 5-7 hours after infection and proceeds exponentially to reach several thousand copies of RNA per cell (45). Three classes of mRNA have been described 35S, 28S and 22S (50). Transcription maps of the MVV genome and the extent of splicing in messenger formation are described in the chapter by Pyper et al. in this book. It is known that the synthesis of virus polypeptides takes place in the cytoplasm in the form of large precursors which are then cleaved into the final polypeptides (52). The precursor proteins include Pr55gag which gives rise to p25, p16 and p14 and Pr150gag-pol which in addition gives rise to the polymerase protein or proteins. Another precursor is the Pr150env which is then cleaved into the gp135 envelope protein. The exact steps in the assembly of the virus are not known but intracellular ribonucleoprotein complexes have been described consisting of viral RNA (35S, 27S, 22S), p25, p16, p14, Pr55gag, gp135 and Pr150env (53). These complexes are infectious for sheep cells, sediment at 120S to 200S and presumably give rise to the fully formed virion by acquiring extra material from the cytoplasmic membrane of the cell in the process of budding from the cell surface.

MVV grows well in sheep cells in vitro. Traditionally workers have used cultivated cells from lamb choroid plexus that look like fibroblasts (Fig. 2A), but other fibroblast-like cells from various organs seem to support virus replication as well (8,54). Macrophages have also been reported to support virus multiplication in vitro (55). Cells from certain other animal species can support virus multiplication especially bovine cells and to some extent human cells. Cells from most other species tested have either failed to support viral replication or produced only minimal titers (56,57,58).

Malignant transformation of mouse cells in vitro by visna virus has been reported (59,60) but other workers have

not been able to confirm any oncogenic potential of MVV (61). Macintyre reported morphological transformation of visna-infected human astrocytoma cells in culture and in sheep cells infected with a strain of virus reisolated from the human cells (58,62). The apparently transformed sheep cells did not grow into tumors after inoculation into transplantation compatible sheep (64). It would be interesting to follow up this work to test if the MVV could pick up oncogenic potential possibly by recombinational events in malignant cells. There is no evidence of oncogenic action of this lentivirus in the natural host (63).

Virus-cell interactions in vivo

Virus can be recovered from various tissues and organs of natural and experimental cases of maedi and visna (54,63). Following experimental inoculation virus can be recovered as early as 1-2 weeks after infection and at any time after that. Titers of free infectious virus are usually minimal and viral isolation often requires the use of tissue explants and blind passages in susceptible cells (54). The virus is present in the central nervous system (CNS), especially in the choroid plexus but also in lymphoid organs, spleen, mediastinal and mesenteric lymph nodes and the lungs. In the peripheral blood the virus is present in an occasional buffy coat cell, apparently only 1 out of 10^5-10^6 cells or so are virus-carriers (14). Free virus has never been found in plasma but during the first 3-4 months following intracerebral inoculation free virus has been found in the cerebrospinal fluid (CSF) (54).

There seems to be a very severe restriction on viral gene expression and formation of virus particles in the infected animal. Thus when using *in situ* hybridization to detect viral nucleic acids Haase et al. found as much as 18% of choroid plexus cells to carry viral DNA (64). When using immunofluorescence they found that only 0.025% of these same cells stained for the p25 main core antigen of the virus.

This restriction disappears when the cells are cultivated in vivo and 14% of these choroid plexus cells were found to contain the complete viral genome by infectious center assay on permissive cells in tissue culture. It has also been very difficult to find budding virus or complete virions in electron microscope studies on tissues from infected animals (65) whereas such particles are numerous in sections from cells infected in vitro.

Cells carrying the visna provirus in the CNS contain in the order of 100 copies of viral DNA. The number of copies of viral RNA however, is greatly reduced compared to permissive cells in vitro and is about two orders of magnitude less (14,66).

There is therefore a restriction of proviral expression at the transcription level in the tissues of infected animals. This restriction does not seem to be caused by immune responses of the host since immunosuppression and fetal thymectomy do not lead to increased virus expression (67,68). Recently it has been reported that MVV and CAEV stimulate the formation of a special kind of interferon (69) the role of which in virus restriction in the host is still unclear.

IMMUNE RESPONSE TO MAEDI-VISNA

Serum Antibodies

Antibodies to virus antigens are induced by experimental and natural infection of sheep with MVV. Such antibodies can be detected using different techniques: virus neutralization (8), complement-fixation (70), immunofluorescence (71, 72), gel immunodiffusion (36, 73, 74), passive hemagglutination (75) and the ELISA method (76). Virus-neutralizing antibodies were first reported by Sigurðsson et al. in 1960 (8). The kinetics of the neutralization process have been described by Thormar (77). Following experimental infection neutralizing antibodies are first detected 1 1/2 - 3 months or even later in some sheep (54,72,78). The titers increase over the next few months and then tend to stay

relatively constant even for several years in sheep that survive that long.

The neutralizing antibodies may be quite strain-specific. Thus some isolates and strains of visna virus show little or no cross reaction with certain antisera (79).

Complement-fixing antibodies first appear 3-4 weeks after experimental infection. They increase rapidly in titer and then tend to stay at a fairly constant level as the neutralizing antibodies. Complement-fixing antibodies seem to be relatively nonspecific not distinguishing between various strains of MVV. This is presumably due to the fact that the crude antigen used for complement-fixation contains the p25 core antigen which has group-specific reactivity, whereas neutralizing antibodies are thought to be directed against the envelope glycoprotein (35).

Precipitating antibodies assayed by immunodiffusion in gels are directed against the virus envelope glycoprotein and against the p25 core antigen. Almost all sheep experimentally infected with the 1514 strain of visna virus develop precipitating antibodies to the glycoprotein but only 40% show a second line corresponding to the p25 antigen. In natural infections with the American progressive pneumonia virus a somewhat lower percentage of sheep will react in these tests (36).

The ELISA method appears to be more sensitive than both complement-fixation and immunodiffusion and seems to be the method of choice for general purposes.

The immunoglobulin class distribution of viral antibodies in maedi visna has not been completely worked out. Complement-fixing and neutralizing antibodies both appear to belong to the IgG1 subclass but can be separated on the basis of electrical charge by ion-exchange chromatography, the neutralizing antibodies being more highly charged (80). There is one report of neutralizing antibody activity in the IgM class (81) but we have been unable to confirm this (80).

Different strains of MVV vary in their abilities to raise neutralizing antibodies. Less lytic strains that grow to a lower titer in tissue culture produce less neutralizing antibodies in sheep than highly cytolytic strains of Icelandic visna virus (36,82, see also chapter by Pyper et al.).

Antibodies to MVV can be raised in other animals than sheep. Hyperimmunized rabbits will produce antibodies detactable by complement-fixation, immunofluorescence, passive haemagglutination or even neutralizing antibodies (75). Monoclonal antibodies to virus proteins can be raised using inbred mice (83,84) and have been useful in detecting antigen-positive cells in vivo (85, see also chapter by Georgsson). Nonspecific inhibitors which are apparently not immunoglobulins have been reported in human sera in low titers and in bovine sera in high titers (56, 86).

Serum and CSF from uninfected sheep contain a heat-stable visna virus-inhibiting factor which is not an immunoglobulin but has not been fully characterized (87).

CSF antibodies

Neutralizing antibodies have been found in the CSF of some experimentally infected sheep and have been shown to be produced locally in the CNS (54,88,89). In some sheep with long-term visna oligoclonal bands in the gammaglobulin region have been demonstrated by electrophoresis (90) but the antigen-specificity of these bands is not known. Plasma cells are seen regularly in the CSF and in inflammatory lesions in the CNS (65, see also chapter by Georgsson).

Increase in the globulin fraction of CSF proteins has been reported in visna (6) and the level of IgM was found elevated but that of IgG1 in the normal range (90). The appearance of neutralizing antibodies in the CSF may coincide with the disappearance of free infectious virus from the CSF 3-4 months after infection (54).

Antigenic variation

It was first reported by Guðnadóttir (91) that a viral isolate from a long-term infected Icelandic sheep could

escape neutralization by the animal's own serum even though the same serum neutralized the infecting virus strain. This phenomenon was confirmed and further studied by Narayan and colleagues in other sheep breeds (92,93). They also showed that antigenic virus variants could be selected in culture by passaging visna virus in the presence of neutralizing antibodies (94,95). Studies of the genomic changes associated with the antigenic variation indicated that the variants were caused by point mutations in the env gene coding for the envelope glycoprotein which is the target of neutralization (35,96,97).

The occurrence of antigenic variation in visna-infected sheep is thus well established. However, in an extensive study of many isolates obtained at different times after infection from a number of long-term infected sheep, variants did not in general replace the infecting virus strain, nor could their appearance be correlated with progression of lesions or development of clinical disease (79, 98). The antigenic variation in MVV is undoubtedly important in creating a whole spectrum of ovine lentivirus strains varying in several biological characteristics such as cytopathic potential for cells in tissue culture, ability to raise neutralizing antibodies in infected sheep, pathogenicity and different tropism for the main target organs in vivo.

Cell-mediated immune response

Our knowledge of cell-mediated immunity to MVV is still fragmentary but several workers have reported blast transformation of lymphocytes from infected sheep in response to virus antigens (99,100,101,102,103). From these studies it appears that the cell-mediated response is irregular, appearing at different times after infection in experimental animals and usually transient. These findings may reflect the limitations of the techniques used. Much remains to be done to clarify the cellular immune response which may be of central importance for the virus-host

interactions and the pathogenesis of lesions which seem to be immmunopathogenetic in nature (67).

Interferon

Earlier studies have indicated that visna virus is a poor inducer of interferon. Thus cell cultures persistently infected with visna virus were susceptible to infection with vesicular stomatitis and vaccinia viruses (104,105). It was also reported that replication of visna virus in permissive sheep choroid plexus cultures was completely unaffected by high concentration of sheep interferon induced by polyriboinosinic-polyribocytidylic acid in fetal lambs or in sheep choroid plexus cultures (105,106). Recently it was reported that lentiviruses of sheep and goats induce a unique type of interferon produced by T lymphocytes during interaction with infected macrophages (69). This interferon is a non-glycosylated protein of molecular weight 54,000-60,000 and is stable to heat and acid treatments. The role of this lentivirus-induced interferon in controlling virus replication and in the pathogenesis of lesions in the infected animal is still unclear (see also chapter by Pyper et al.).

Vaccination attempts

Limited attempts have been made to vaccinate sheep against MVV (107). The sheep were inoculated with whole virus inactivated with UV-irradiation and mild heat treatment and mixed with complete Freund's adjuvant. The inoculated animals developed antibodies that could be detected by complement-fixation, gel immunodiffusion and ELISA but no neutralizing antibodies. On challenge with live virus by the respiratory route no protection against infection was found. Similar negative results were obtained using the envelope glycoprotein gp135 of the virus concentrated from tissue culture supernatant and partly purified on lectin columns. Cutlip et al. reported similar failures of experimental vaccines against progressive pneumonia (108). Although these first attempts of vaccination against lentivirus infection have not been

successful further efforts should be made. In view of the threatening epidemic of human AIDS, animal lentivirus infections such as maedi-visna may be used as models for various approaches to prevention. There are several aspects of lentivirus infections that may present difficulties in obtaining a protective vaccine. Since the viruses may hide inside cells in a provirus-like form without expression of viral proteins, neutralizing antibodies may be ineffective in preventing infection carried by cells in secretions or blood. The lesions of visna are believed to be immunopathogenetic in nature since they are abolished by immunosuppressive treatment (67). Furthermore immunization with lentiviruses may enhance the severity of disease (109,110). In a more optimistic vein, we have suggestive evidence that virus-neutralizing antibodies appearing early after initiation of experimental infection may result in less severe disease later on (G. Pétursson et al. unpublished observations).

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5

DIAGNOSTIC METHODS

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ABSTRACT

Maedi-visna virus (M-VV) classified as a lentivirus, an exogenous, non-oncogenic subgroup of the retrovirus causes a characteristic disease in sheep, but neither the clinical signs nor the pathological and histological findings are pathognomonic for the infection with M-VV. In the infected animal virus is found in the form of proviral DNA; few cells are infected and direct detection of virus in naturally infected sheep is not possible. At present, diagnosis of natural infection in a flock of sheep is confirmed by detection of antibodies against M-VV, by AGID or ELISA.

INTRODUCTION

Maedi-visna virus (M-VV) causes a persistent infection. Disease is associated with a chronic progressive proliferation of lymphoid tissue in lungs, brain, udder and joints, but many infected sheep remain in a subclinical phase of the disease. In spite of a cellular and in most cases a humoral immune response the animal never gets rid of the infection.

The clinical signs of M-VV infections: laboured breathing, illthrift, wasting in spite of a good appetite, udder induration, hind limb paralysis, and in some cases swollen joints with or without lameness should give rise to suspicion of the infection, but neither the clinical signs nor the pathological and histological findings (see chapters 2 and 3) are pathognomonic for the infection with M-VV. The diagnosis has to be confirmed either by detection of M-VV (virus particles, proviral DNA, viral RNA, viral proteins) or (as sheep infected with M-VV are persistently infected) by detection of antibodies against M-VV.

DETECTION OF THE VIRUS OR VIRAL ANTIGENS

M-VV causes a persistent infection characterized by a severe restriction of viral replication: only a small number of cells is infected, many

of which carry the viral information in the form of proviral DNA.

The restriction for viral replication is not present when infected cells are cultivated in vitro. Here budding particles can be detected by electron microscopy and viral proteins be demonstrated. It is, however, very, very rare to detect viral particles by examination of sections from naturally infected sheep (1).

Due to the low number of infected cells and the restricttion in vivo for virus replication diagnosis of M-VV infection in naturally infected sheep cannot be established by direct detection of viral particles, nucleic acids or proteins. The existence of individual animals in which M-VV can be isolated but no antibodies be demonstrated is also well known. Diagnosis of individual suspect clinical cases has, therefore, as yet to rely on virus isolation in cell culture.

Virus isolation from buffy coat by co-cultivating with permissive ovine cells, e.g. choroid plexus cells, can be attempted from living suspected sheep, but one should bear in mind that only one in 10^6-10^7 leukocytes contain the viral information, thus a negative result does not exclude that the animal is infected (2).

Following necropsy, virus isolation from choroid plexus, spleen, mediastinal lymph node, lung, synovial membrane or mammary glands by co-cultivation is normally successful. The cytopathic effect is characteristic and consists of stellate cells and multinucleated giant cells. Some American isolates, however, are only slightly cytolytic and behave more like Caprine Arthritis-Encephalitis Virus CAEV in cell culture.

Choroid plexus cells from fetal or newborn M-VV-free lambs can be used for co-cultivation up to 15-20 passages. In higher passage number, the susceptibility to virus replication is reduced even though the cells look and grow well. M-VV can replicate in other cells like ovine testis cells, ovine lung fibroblasts, caprine choroid plexus cells, caprine endothelial cells, and synovial membrane cells.

After inoculation the cultures should be kept for two weeks before passaging. If not kept in CO_2 atmosphere the addition of Hepes buffer to the medium is recommended. As some field strains are not cytolytic or only weakly cytolytic, all cultures should be investigated by immunofluorescence or immunoperoxidase methods for the detection of viral antigens after the second blind passage.

Various methods can be used to identify the isolated virus, e.g. elec-

tron microscopy, immunolabelling of viral antigens in cell culture and detection of viral antigens by ELISA. Due to variability of the envelope glycoprotein, neutralization tests may not always be a relevant tool.

In experimentally infected sheep proviral DNA and viral RNA have been demonstrated by in situ hybridization in foci of inflammation in the brain, and a very low percentage of cells carrying viral nucleic acids M-VV proteins gp135 and p25 have also been demonstrated by an immunoperoxidase method. The sheep received 10^9 PFU M-VV strain 1772 by intercerebral inoculation (3, 4).

The strains of M-VV found in naturally infected sheep flocks may be less pathogenic, the number of infected cells may also be less compared to the situation in experimentally infected sheep and so far the diagnostic methods have not been suited for detection of viral nucleic acids or viral proteins in naturally infected sheep. Amplification of proviral DNA is needed for detection and the new polymerase chain reaction, PCR (5) may in future increase the diagnostic possibilities.

DETECTION OF CELL-MEDIATED IMMUNE RESPONSE

Evidence of cell-mediated immunity in infected sheep has been obtained by the lymphocyte-blast transformation test (6, 7, 8). The cell-mediated response seems to be irregular and not suitable for diagnostic purposes although the reaction may be important for understanding the pathogenesis of lesions due to M-VV infection.

DETECTION OF ANTIBODIES AGAINST M-VV

From a practical point of view the only diagnostic methods to use in living animals are the serological assays for detection of antibodies against M-VV.

The majority of animals respond to infection with M-VV by production of antibodies, but the length of time from infection to seroconversion is unpredictable and may take months or even years. Furthermore, some infected animals remain antibody negative. Consequently, negative test results of the investigation of sera from individual animals cannot be used to ascertain freedom from infection with M-VV.

The disease is always introduced to a clean flock by an infected animal, and purchase of sheep should be restricted to animals from M-VV-free flocks.

A flock of sheep can be declared free from infection with M-VV if it has or has had no contact with infected sheep and if it passes three serological tests with intervals of 6-12 months without showing positive reactions.

The serological methods to be used in these screening programmes can be the agar gel immunodiffusion test (AGID) or enzyme-linked immunosorbent assay (ELISA).

The complement fixation (CF) test was described in 1967 by Gudnadottir and Kristinsdottir (9), and the test has been used in the Icelandic and Norwegian surveys. A microplate CF test was used during the first years of the Danish control programme for lentivirus infections in sheep and goats. Flocks classified as free from infection by repeated CF tests did not change status when the CF test was replaced by the AGID test using antigen containing gp135 of M-VV. In infected flocks, however, the number of positive animals in some cases was increased when screening was performed by the AGID test.

In order to avoid problems with the anticomplementary effect it is recommended to pretreat sera with equal amounts of complement for 1 hr at 37°C followed by inactivation for 30 min at 56°C. A modified CF test using addition of c_1q in the form of bovine serum has been reported (10).

The simplicity of ELISA and the AGID test makes these methods better suited than CF test for screening programmes.

The AGID test was first described by Terpstra et al. (11). At that time it was not specified whether the antigen contained gp135 or p25 and only one precipitation line was obtained. An AGID test with two precipitation lines was later reported (12). It has now been demonstrated that the majority of infected sheep have antibodies against gp135 in the AGID test. A minor group of these animals shows additional antibodies against p25 in the AGID test, whereas very few sheep only have precipitating antibodies against p25.

The AGID test using antigen containing gp135 has proven to be a simple and reliable flock test. Agar or agarose can be used for the test plates but it is important to include 8-8.5% sodium chloride in the 0.05 M Tris buffer pH 7.2 when making the gel. Concentrated supernatant fluids of infected cells are the most inexpensive antigen; a 50-100 x concentration of cell culture fluid is needed. To get good reproducible results it is necessary to standardize the the content of gp135 in the antigen. Testing of a weak positive serum or dilutions of a positive serum in negative serum should

be included in the standardization of each antigen batch.

The identity of precipitation lines of control sera should be ascertained by testing the sera against purified gp135 and p25. Thus the weak positive control serum should react only to gp135 whereas the positive control included in each run should react to both, ensuring that antibodies of test sera to p25 will also be detected (13, 14).

The AGID test with antigen containing gp135 is a simple, reproducible and specific diagnostic method well suited for screening purposes. Reading the plates is subjective, cannot be automated, and experience is required. The advantage of the different ELISA modifications is that many steps in the procedure can be automated and the results can be calculated in an objective way.

The first ELISA procedure reported was an indirect ELISA using disrupted, pelleted virus as antigen coated directly to the microtiter plate (15). The sensitivity of the indirect ELISA was shown to be better than the sensitivity of the AGID test. However, the specificity was less. The number of false positive reactions obtained in indirect ELISA varies depending mainly on the quality of the antigen preparation. For experimental work, indirect ELISA using purified M-VV proteins has been applied, but the cost of antigen in such assays is prohibitive for use in large screening programmes.

Recently, an ELISA using two monoclonal antibodies against p25 has been reported (16). The test is named complex trapping-blocking ELISA (CTB--ELISA). One of the monoclonal antibodies is used as catching antibodies and the other - directed against a different epitope on p25 is enzyme-labelled and serves as detecting reagent. ELISA methods detecting p25 antibodies will be advantageous if vaccination should be commonly used. The drawback of the p25 ELISA assay is that conformatory tests are likely to be gp135-specific (e.g. AGID test which is known to be highly specific).

The indirect immunofluorescence test has been used to demonstrate antibodies against M-VV (17, 18). Comparative studies of the immunofluorescence test, AGID test with antigen containing gp135, and conventional indirect ELISA have been performed (19). The results showed that the tests had more or less similar diagnostic sensitivities. The involvement of cell culture work in connection with the immunofluorescence test makes the test less suited for mass screening purposes.

M-VV causes fusion of cells fusion from within is a result of virus replication in the cells, fusion from without requires no viral replication

and occurs shortly after inoculation also with inactivated virus.
The biological function of fusion from without is not known, the fusion activity can be blocked by treatment with antibodies against gp135. The principle is not used for a routine test.

The neutralization test for M-VV antibodies requires a long incubation time for the serum-virus mixture (20). Incubation periods of 18-48 hr at 4°C are recommended. Neutralizing antibodies develop relatively late in the course of infection compared to antibodies reacting in other tests (21).

The interest in neutralizing antibodies is connected to a possible protective effect of such antibodies, but so far no inactivated vaccine has been proved to induce neutralizing antibodies.

Neutralizing antibodies have been used to characterize different M-VV variants (see chapter by Pétursson), but although the strain specificity of neutralizing antibodies excludes the neutralization test as a broad diagnostic method, cross neutralization is often obtained with sera from naturally infected sheep (22).

CF test using supernatant fluids from infected choroid plexus cells as antigen, AGID test with antigen containing gp135, indirect ELISA with pelleted virus as antigens and CTB-ELISA with monoclonal antibodies against p25 do not discriminate between strains of the virus.

Diagnosis of M-VV infection in naturally infected sheep can at present be summarized in the following manner.

Category of animals	Negative test result not reliable	Negative test result valid
Single living animal	Clinical symptoms Virus isolation from blood Serology	PCR ?
Single dead animal	Pathology Virus isolation from organs Serology	PCR ?
Sheep flock		Consecutive Serology

Positive test results: Virus isolation and/or demonstration of antibodies against M-VV indicate that the flock of origin should be regarded as infected until proved negative.

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6

ECONOMIC IMPORTANCE, EPIDEMIOLOGY AND CONTROL

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ABSTRACT

This chapter covers economic importance, epidemiology and possibilities for the control of maedi-visna and related ovine lentivirus infections, following an historical introduction in conjunction with an outline of their geographic distribution.

GEOGRAPHIC DISTRIBUTION

INTRODUCTION

A complete picture of the geographic distribution of ovine lentivirus (OLV) infections, e.g. maedi-visna virus (MVV) and ovine progressive pneumonia virus (OPPV), with their incidence in the respective sheep populations cannot be given. However, the information available so far indicates that Oceania is the only major geographical area of the world which can be considered OLV-free. This is probably due to the fact that the sheep in this region descended from small numbers of early 19th century imports which were apparently free from OLV. An isolated geographical position and severe restriction on further importations in the 20th century subsequently prevented virus introduction. It is of interest to note in this respect that in the Australian dairy-goat population, the goat lentivirus (CAEV) is wide-spread whereas the feral goat population, which probably originated from earlier importations, appears to be virtually free from CAEV (1, 2).

The ancestors of today's sheep were probably domesticated in the Middle East approximately 10,000 to 7000 years BC and gradually moved overland to Europe and Northern Africa, and from there eventually overseas to the New World with the settlers. The work of Bakewell and Ellman in Britain at the end of the 18th century, providing the basic material for the modern meat breeds, and the contemporary emergence of the fine-woolled Merino along with the rise of the wool industry, greatly enhanced the movement of sheep throughout the world.

When analysing the present situation with respect to the distribution of OLV, it seems unlikely that the movement of sheep in the 19th century significantly contributed to the spread of the infection. The British meat breeds in particular were exported to many areas of the world and eventually developed into local variaties or new breeds. They were probably free from OLV as Britain has only acquired the infection in the last decades through, ironically, importation of breeding sheep. The Merino originally moved from Spain to Sweden, Holland, England, Germany, Russia, North America, Argentina and Uruguay. From Holland the Merino was taken to South Africa and from there it reached Australia, where a few years later Merinos from Germany and Britain also landed. In the second half of the 19th century Australia also imported Merinos from North America. As the sheep populations of Australia and New Zealand still appear to be free from OLV it seems likely that the original sources of the sheep were OLV-free too, or at least not heavily infected. Consequently, this suggests that OLV has become relatively widespread in the second half of the 19th century or later. On the other hand, the first note on a condition described as "broken wind" in sheep in the Netherlands is given in an agricultural report in 1862. This may have been OLV-disease, as sheep pulmonary adenomatosis (SPA) was never indigenous in the Netherlands as far as we know. In South Africa, descriptions of chronic lung conditions date even further back (1825, 1837), but here confusion with SPA cannot be excluded (see below).

The question as to where and when OLV initially came into existence is open to speculation. It could be suggested that a more or less simultaneous emergence of OLV occurred at different locations in the world after the beginning of the major movements of sheep. From such initial focuses the infections could then have spread further through regional movement of infected sheep. On the other hand, it could also be hypothesized that the origin of OLV dates further back in time, with one source from which the virus spread over the world together with the modern sheep breeds during the 19th century followed by further local evolution. The changes in sheep husbandry of the last hundred years in conjunction with changes in genetic composition of the sheep may have facilitated clinical expression of the infection, and subsequently its discovery. Both options could explain the differences between the ovine lentivirus we know today, e.g. MVV, OPPV, Israeli ovine lentivirus and the recently described South African virus (see further). It is clear, however, that man played a crucial role in the distribution of the infection.

The question as to how OLV came into existence also remains open. Accidental combination of quiescent retrovirus genes present in the genes of the different breeds which were crossed may offer an explanation. Another possibility is a non-ovine virus reservoir and here one first inclined to think of goats with their closely related lentivirus CAEV and secondly of horses with their lentivirus, equine infectious anemia virus (EIAV). However, it is not known whether CAEV and/or EIAV are 'older' than the ovine lentiviruses or whether these lentiviruses have a common ancestor in another, as yet unknown, species.

A comparable situation with questions of a similar nature and a documented variety of genetically different strains, some of which probably with different pathogenic characteristics, is currently evolving with the human lentivirus, HIV.

The world sheep population is at present estimated at approximately 1,122 million and its gross global distribution is given in the following table.

Asia	311 million
Oceania	220 "
Africa	193 "
USSR	143 "
Europe	133 "
South America	102 "
North/Central America	19 "

source: FAO Production Yearbook 1984.

The figures illustrate that the density of the sheep population differs greatly throughout the world. The degree of industrialization of sheep production in different parts of the world varies considerably.

In general, sheep were kept to produce meat and wool for the local/national market with the exception of Australia, New Zealand and Uruguay where the fine wool for the world market was the main product. Lamb, the former by-product, became increasingly important as the economic value of wool came under pressure due to the competition of synthetic fibres. Along with the development of modern meat conservation and transport techniques, sheep production is gradually changing from a locally orientated industry to a global enterprise where new markets are feverishly explored and production systems

are intensified. These developments often call for rapid genetic improvement of (meat-)production traits and thus in many cases for importation of foreign breeds.

Intensification as such, especially introduction of temporary housing (indoor-lambing), in conjunction with importation of infected foreign stock in hitherto OLV-free populations and/or regional movements of sheep, create the situation where OLV gets the chance to exhibit its pathogenic potential to full extent.

GEOGRAPHIC DISTRIBUTION

A few countries with a particular history as regards OLV will be mentioned in some detail in more or less chronological order, and other countries will just be mentioned to give an indication as to what extent OLV is recognized in the world. Clinical, pathological and eventually virological examinations were initially used to establish the presence of OLV, but later, when serological techniques were available, detection of specific antibodies was mainly used for this purpose.

South Africa

The first pathological description of a condition in the lung of sheep which fits the picture of maedi or progressive interstitial pneumonia was given by Mitchell (3) in 1915. Later, De Kock (4) described a similar condition which was designated Graaff-Reinet disease after the experimental station where it was found. Initially, there was considerable confusion concerning the distinction from SPA and it took several years before the difference was definitely established. A recent publication describes the isolation of a lentivirus which is antigenically closely related with OLV and a limited survey suggests wide-spread occurrence of this virus, but without lesions (5). The authors point out that OLV-lesions have not been observed since De Kock described Graaff-Reinet disease. A similar observation was reported previously (6). If these results can be substantiated, this lentivirus could then present the first established non-pathogenic OLV. Such a finding would be of considerable scientific interest and would stimulate the search for such viruses in other parts of the world where the pathogenicity of local OLV-infections is questioned.

U.S.A.

Marsh (7, 8) described a chronic interstitial pneumonia found in Montana and other northwestern states which had been observed since 1915 and was designated Montana sheep disease. The etiological agent, ovine progressive pneumonia virus (OPPV), was isolated some fifty years later (9). Serological surveys have shown this virus to be widely disseminated in the sheep population with local variation in seroprevalence between 1 and 70 per cent (10, 11, 12). Other reports have shown similar levels in selected flocks. In general, losses were thought to be low. This led some authors to assume that the pathogenicity of OPPV was low in comparison with Icelandic MVV (11).

Iceland

For several reasons Iceland takes a special position in the history of OLV-infections. Icelandic research workers were the first to isolate causative agent from the pathological entities visna and maedi (13, 14), which were later found to be different manifestations of the same virus infection. They identified the dramatic effect the disease had in their sheep population (15) and were the first to recognize the peculiar character of the infection. This together with observations on scrapie, SPD and paratuberculosis, led Sigurdsson (16) to postulate his, now classical, concept of 'slow (virus) diseases'.

Iceland was also the first country which managed to eradicate the infection through a heroic stamping-out policy maintained over a period of almost thirty years (15). The general acceptance of the Icelandic terms maedi (dyspnoea) and visna (wasting) as the international designation of OLV is a tribute to the Icelandic workers for their far-reaching contributions to our current knowledge.

The use of the term OLV is presently proposed to incorporate and acknowledge the recent view that different strains of ovine lentivirus, possibly with different pathogenic properties, exist.

The Netherlands

The first account of a chronic pulmonary condition in sheep in the Netherlands dates back to 1862 given by Loman (17). Koens (18) described the clinical signs and the pathology of the disease called 'zwoegerziekte' and noted the resemblance to a disease described in South Africa (3) and Monta-

na sheep disease (7, 8). De Boer (19) isolated the virus, demonstrated the similarity of this virus with Icelandic maedi virus, transmitted the disease with tissue culture propagated virus, and in another study (20) showed that his isolate was also capable of inducing a leucoencephalomyelitis (visna).

A serological survey demonstrated widespread infection of the Dutch sheep population (21).

France

In 1943 Lucam (22) described a disease of the lungs of sheep locally known as 'la bouhite' and concluded that this condition was probably identical with Graaff-Reinet and Montana sheep disease. Later, the presence of OLV was confirmed by demonstrating specific antibodies in suspected sheep (23) and the causal virus was isolated by Russo et al. (24). Serological surveys have revealed the prevalence of OLV in many regions and different breeds in France (25).

Other European countries

Besides the Netherlands and France, OLV-infections have been reported in most other European countries. In the G.F.R., the disease was reported and the virus isolated (26, 27) and a serological survey showed the infection to be wide-spread (28). The G.D.R. also reported the disease (29). In Belgium, a high seroprevalence among Texel sheep was recorded (30). The disease was diagnosed in Swiss sheep (31). The United Kingdom has probably only fairly recently imported the infection with foreign breeds (32). The Irish Republic imported the virus in the sixties but has since managed to control and probably eradicate the infection (33). The Scandinavian countries have also reported the occurrence of OLV-infections, i.e. Denmark and Norway report connection with importation of Texel sheep (34, 35, 36). In Sweden the origin of the infection is unknown (37).

In Greece OLV-disease was described (38) and serological data imply a considerable incidence of infection (39). Italy has recently reported the presence of the disease, the prevalence of infection in its sheep population and isolation of the virus (40, 41). Spain also reported pathological and serological evidence of OLV (42). Furthermore, in Bulgaria (43), Hungary (44) and Poland (45, 46) the pathological lesions have been recognized. In Rumanian sheep slaughtered in Israel, the lung lesions were recorded (47).

Middle East

Pathological evidence of OLV-disease has been found in Turkey (48). In Israel, OLV-antibodies and lesions were found (49) and virus was isolated (50).

A recent study using nucleic acid hybridization indicated extensive sequence homology of the Israeli virus isolate with OPPV and relative dissimilarity with other members of the lentivirus group including Icelandic and Dutch OLV (51). Antigenic differences were also observed (52).

USSR

Little information is available on the situation as regards OLV in this large sheep population. Only one report mentions the occurrence of the disease in Kirgizia (53).

India

Several reports have been published on the incidence of OLV lung lesions in slaughterhouse material from different states (54, 55, 56, 57). The results indicate the presence of OLV in many parts of India.

China

One report so far mentioned the occurrence of OLV-lesions (58).

Africa

Besides Graaff-Reinet disease (4) in South Africa, OLV-associated disease and antibodies were described in Kenya (59).

In Morocco, serological evidence of OLV in imported sheep from Europe was recorded (60). A serological survey performed in Nigeria demonstrated a low but wide-spread incidence of OLV-antibodies (61). A recent report supplies data on the serological prevalence in Algeria (62).

North and South America

As well as in the USA, OLV has been shown to occur in Canada (63, 64) and a serological survey indicated a considerable incidence (65).

It is of interest to note that a serological survey among the wild Bighorn sheep in California revealed no antibodies to OLV (66).

Histological lung lesions suggestive of OLV were described in Mexico (67).

In Peruvian sheep, antibodies and lesions were reported (68, 69).

ECONOMIC IMPORTANCE OF OLV-INFECTIONS

General economic damage

The clinical and pathological manifestations of the OLV-disease-complex are interstitial pneumonia, indurative lymphocytic mastitis, leucoencephalomyelitis, arthritis and vasculitis, which may occur together or singly in individual animals. All features are characterized by a slow progressive course.

Leucoencephalomyelitis is a relatively uncommon manifestation which is mostly observed in single animals. Chronic arthritis and vasculitis have been reported to occur with a considerable incidence in association with OPPV-infections (70, 71), but such observations have not (yet) been made in natural MVV-infections. In this respect, it is important to note that genetic, antigenic and biological differences between OPPV and MVV have been reported, which supports the notion that different pathogenic properties could exist (72, 73, 74, 75).

As the disease-complex does not 'strike' in a flock, but develops insidiously and slowly over the years, the uninformed shepherd will often already have accepted a high culling rate without being aware of its cause. In other words, flock management will adapt to the situation by increasing the replacement rate. Even the smallest increase in replacement rate over many years can amount to considerable financial damage. In addition, it should be noted that the situation in a flock or population is usually not static but progressive, as the infection will continue to spread resulting in an increasing incidence of infection and, consequently, disease.

Assessing the financial damage caused by OLV-infections is a complicated task as it is associated with factors such as husbandry, management and the structure of the local sheep industry. This implies that it can practically only be calculated on the basis of comparing the financial results of large number of infected and non-infected flocks. Such studies have not yet been undertaken and hence very little accurate information is available.

Iceland set an extreme example; the infection turned out to be a disaster for the sheep industry. The husbandry situation in Iceland was, how-

ever, also extreme and facilitated this disastrous effect (15). In the Netherlands, with its moderate sea climate, König (76) performed a comparative general flock health study comprising OLV-infected as well as OLV-free flocks and concluded that OLV-induced interstitial pneumonia was the most important sheep disease in economic terms. A possible effect of the indurative lymphocytic mastitis was not included in this study.

The overall financial damage in a flock can be broken down to the following aspects.

Direct effects:

- Loss of sheep which succumb to the disease-complex, or sheep which are forcibly culled in an advanced stage of emaciation with almost negligible slaughter value. In flocks with a reasonable standard of management this will hardly occur as affected sheep are culled in an early stage of disease. Ewes developing leucoencephalomyelitis are more easily recognized and can be sold for slaughter in an early stage, although the body condition of such animals is usually already poor.
- Increased culling rate, consequently increased replacement rate. Early signs, loss of body condition in most cases, are hard to assess without close inspection. Ewes with a poor body condition at inspection are usually culled. This also applies for ewes with an indurated udder or chronic arthritis. The clinical effect of vasculitis is as yet unknown. Ewes which produced poorly growing lambs are prone to culling as well, and this may be associated with the presence of indurative mastitis.
- Decreased pre-weaning growth rate of lambs as a result of indurative lymphocytic mastitis in the ewes (see next section).

Indirect effects:

- According to the available information, the immune system does not appear to be compromised by OLV-infection. It is likely, however, that animals with lesions, particularly in the lung, are prone to secondary (bacterial) infections. Concurrence of parasitic infestation and OLV-lesions may be expected to aggravate the clinical picture.
- Lower market value of breeding sheep from infected flocks as soon as the market demands OLV-free production or breeding stock. This aspect of the financial damage obviously depends on developments in the trade.

- Reduced chance of success on the international export market of breeding sheep as freedom from OLV is being incorporated in the import conditions of an increasing number of countries.

In flocks with a reasonable standard of management, the major aspect of the damage undoubtedly are the increased replacement rate and decreased pre-weaning growth of the lambs, both of which directly affect flock performance. Even with regular flock inspections, the culled sheep represent a lower than slaughter value which results in an extra financial loss.

The direct effect in terms of loss of sheep depends on the management, i.e. more frequent flock inspections results in earlier detection of affected animals and at the same time prevents further loss of slaughter value. The indirect effects are generally of little economic importance, but breeding flocks may suffer from a decline in sales.

The degree to which the replacement rate will increase depends on several factors. If, e.g., the replacement rate is already high for other reasons, the average age of the sheep in the flock will be low and OLV-disease will hardly be found as it mostly affects animals over three to four years of age. Consequently, in this situation the replacement rate will hardly rise. Furthermore, the level of infection in the flock is also important as disease in most cases only becomes noticeable when a considerable percentage of the sheep (> 30%) is infected. Thus, when the disease becomes overt in a flock level of infection will already be considerable.

Damage due to OLV-induced mastitis

Indurative lymphocytic mastitis is a relatively recently discovered feature of the OLV disease-complex which is probably even more concealed in its clinical presentation than the other features. It probably directly affects one of the major traits of a ewe: the production of milk. Especially in the modern breeds with increased lambing percentages, the milk producing capacity of a ewe is of crucial economic importance.

In a study on vertical transmission of OPPV, Cross et al. (77) described typical lesions in the udder of an OPP-affected ewe and noted the morphological resemblance with the lung lesions. Griem and Weinhold (78) in their detailed study of the morphological changes in OLV-affected ewes in Germany described similar lesions. A higher incidence of an homogeneous firmness of the udder on palpation in OPPV-seropositive sheep was noted by Huffman et al.

(12), but the experimental design did not permit etiological conclusions. Oliver et al. (79) observed a massive lymphoid proliferation in the mammary gland of a few of their seropositive sheep suggesting etiological involvement of OPPV. Also in the USA, Cutlip et al. (80) found this type of lesion in 14 of 18 (77 per cent) experimentally OPPV-infected sheep 2.5 to 10 years post-inoculation. Although the common causes of mastitis could be excluded, the etiology was not proven. Similar observations were presented by Deng et al. (81). In the Netherlands, a high incidence of palpated udder induration was reported in four flocks with problems of poor lamb growth and a high OLV-sereprevalence, whereas four OLV-free flocks without such problems showed almost no induration (82). At necropsy of a number of ewes from both groups, an indurative lymphocytic mastitis was observed in the ewes of the infected flocks.

Thus, circumstantial evidence for etiological involvement of OLV accumulated, but there was still no indisputable evidence. However, Van der Molen and Houwers (83) presented definite proof. Eight primiparous ewes, intravenously inoculated with cell culture propagated MVV, showed the typical lesions when slaughtered at eight, 16 and 24 months after inoculation, whereas the sham-inoculated controls kept on an adjacent pasture did not show lesions. Lung lesions were also observed, but to a lesser degree.

Consecuently, there is substantial evidence that the indurative lymphocytic mastitis is an intrinsic feature of OLV-infections. It is not clear why this feature has been recognized only recently, but it could be argued that this is merely the result of pre-focused attention on lung and brain.

As to the question of incidence of these udder lesions, information is stille limited. Houwers and Van der molen (84) found a high incidence of udder lesions in a study on natural transmisson. Of a total of 20 ewes which had become infected during the course of the experiment, eight had udder lesions only, three only had lung lesions, while another three showed lesions in both organs at necropsy, yielding a total of 55 per cent udder lesions against 30 per cent lung lesions. The incidence of 77 per cent found by Cutlip et al. (80) is particularly significant. Together with the clinical observations of high incidences of induration, the data available so far suggest that the indurative lymphocytic mastitis may well be the most prevalent lesion of OLV.

The paramount question is what effect these lesions exert on milk pro-

duction. As yet, information is limited. In addition to the reported poor lamb growth (82), one report described lamb mortality associated with udder induration (85) which can retrospectively be associated with OPPV-infection, and another describes unavailability of milk in periparturient seropositive ewes (86). Given the severe induration of the udder tissues, which is associated with a gradual disappearance of secretory tissue, it seems inevitable that the milk producing capacity of the udder is affected. Udder lesions may develop earlier than eventual lung lesions (83) and considering the recorded high incidence, both clinically (82) and pathologically (80, 81, 84), the effect on milk production in general might be severe.

It is tentatively concluded that the indurative lymphocytic mastitis will reduce the milking potential of the ewe, generally resulting in poor pre-weaning growth and possibly even in an increased mortality rate of the lambs (85), especially of twins and triplets. The gross output of an infected flock will thus be affected. Poor pre-weaning growth is unlikely to be compensated for in the post-weaning period and only at the cost of considerable extra food intake.

It should be noted that in cases of this mastitis, the available milk has a normal appearance.

General conclusions

The degree of economic damage caused by OLV-infections is associated with the type of sheep production and the local husbandry situation, and is thus variable. Intensive production systems, especially with winter housing and/or indoor-lambing, facilitate spread of the infection and hence the clinical and subclinical expression of disease. Figures of annual financial losses in individual flocks attributed to OLV-disease are rare. Especially figures, which reflect the average situation and which can be translated into gross economic terms, are lacking.

The general economic damage should be expressed in terms of increased replacement rates and reduced output of lamb weight per ewe. The overall effect of OLV-infection in a flock or population is usually not dramatic in terms of recognizable disease but takes its toll in terms of chronincally reduced flock performace, and thus in reduction of net income per ewe per annum. Its degree depends on various flock/population associated circumstances. In addition, it should be emphasized that OLV-infection in a flock is usually not a static event, but progressive, leading to increasing levels of

infection with time and, consequently, increasing incidence of disease. On the other hand, it is possible that an equilibrium is reached between the usually slow dynamics of spread and counteracting forces, e.g. a fortunate selection policy in conjunction with adequate culling (see later section). Husbandry conditions which do not specifically support horizontal transmission can also be considered counteractive. It is therefore likely that a static situation will establish itself in most flocks sooner or later, be it at the cost of a reduction in net income per ewe per annum. However, in particular situations, as exemplified by the Icelandic experiences, OLV may become a severe threat to the existence of the sheep industry (15).

The general tendency in the sheep industry towards further intensification will lead to situations which predispose to considerable damage caused by OLV-infections.

EPIDEMIOLOGY

Breed susceptibility

As yet, it is unclear whether there is genetic predisposition to contracting an infection with OLV. However, preliminary results presented by Dawson (87) and a recent study by Houwers and Visscher (8) provide strong indication for a breed-associated difference in susceptibility to infection.

A genetic prediposition to expression of disease has been suggested on the basis of observations on the incidence of infection and disease in different breeds. OLV usually appears to be introduced in a flock or geographically restricted population by the introduction of infected sheep. Although rams are most commonly used for introducing new genes, ewes are more efficient in establishing the infection in a flock (see later section). If ewes are introduced, they and their progeny are usually kept as long and in as large a number as possible. Depending on the practice of management and husbandry, the infection will in most cases only slowly spread to other sheep but it is probably efficiently transmitted to the progeny of infected ewes, thus creating a situation where the infection remains mainly restricted to the progeny of the originally introduced ewes. This pattern may last for several generations before it resolves or becomes less rigid, and consequently when pure-breeding is involved it may erroneously be concluded that the introduced sheep are most susceptible than the indigenous breed. A similar conclusion can be obtained when the female cross-bred progeny of the

introduced ewes are kept for production. This basically misleading pattern, together with the varying management conditions in general, make it very difficult, if not impossible, to come to justified conclusions in this respect.

However, epidemiological studies in Iceland indicated that the expression of disease was delayed, but not halted, in cross-breeds of ewes of the local breed with Border Leicester rams (15). Experimental intrapulmonary infections suggested the Icelandic breed to be more susceptible to development of lesions than Texel sheep (19). Fairly conclusive evidence was recently provided by Cutlip et al. (89) who, in a retrospective study, showed a consistent difference between Columbia and Border Leicester sheep after experimental as well as natural infections with OPPV, i.e. significantly more Border Leicester sheep developed lesions and the lesions were more severe. In addition, their data suggest that the realtive resistance of Columbia sheep was not associated with the use of a certain virus strain.

It can thus be concluded that breed-associated predisposition to infection as well as expression of OLV-induced disease appear to exist, but that complete breed-associated resistance has, as yet, not been demonstrated.

Modes of transmission and infection

Comparatively little information is available regarding the modes of transmission of OLV. However, from experimentally obtained information and probabilities based on circumstantial evidence, a tentative picture can be drawn. A primary question is where the virus resides in the host. Virus can be isolated regularly from the blood of infected animals and was shown to be almost entirely associated with the white blood cell (WBC) fraction (19, 90). A more recent study has shown a very small minority of the leukocytes (1 in 10^6 to 10^7) to harbour the virus (91). Other investigations have demonstrated that the monocytes among the leukocytes contain the provirus and when these monocytes mature to macrophages, replication of the virus is initiated (75, 92, 93, 94). This suggests that especially organs with large numbers of macrophages, e.g. lung and udder, can be sources of virus and may therefore play a role in the epidemiology. The lung can thus be expected to excrete cell-free or cell-bound virus and this is in accordance with the general view that aerogenic transmission is an important route. As "droplet-infection" is most likely involved, this could explain

why housing of sheep appears to be a particular important factor in the epidemiology (see next section). Dawson et al. (96) found enhanced horizontal transmission in housed sheep in association with a case of concurrent SPA. The abundance of alveolar macrophages in the SPA-affected lung was probably a rich source of OLV and the fact that the sheep were housed offered favourable conditions for aerogenic transmission. Enhanced transmission of OLV in flocks in association with concurrent SPA has also been observed by others (97, 98).

A question of considerable epidemiological importance is whether development of lesions in lung and udder coincides with increased production of virus in these organs with subsequent increased excretion of virus. If this is the case, the degree of transmission could be enhanced. Petursson et al. (91) demonstrated a clearly positive association between the frequency of virus isolations and the severity of brain lesions after experimental intracerebral inoculation of a series of sheep. As yet, it is unclear whether this applies for organs such as lung and udder. In view of the increased number of macrophages in affected lungs and udders such an effect would, however, be likely.

Secondary infections could also have an enhancing effect on transmission, on the one hand through an increased number of macrophages (more virus) and on the other, through induction of coughing (aerosols).

Epidemiological observations indicate that indirect transmission of the virus through contaminated sheds and premises is rare (15). Experimentally, the infection has possibly been transmitted with drinking water contaminated with faeces from infected sheep (99). However, isolation of the virus from faeces has never been reported and reports on isolation from urine are inconsistent. The virus has been isolated infrequently from saliva and nasal swabs (19, 100). It is unlikely, however, that the virus remains infectious for long periods of time outside the host, which could explain why indirect transmission through utensils contaminated with faeces or other excretions has not been observed. Consequently, significant transmission is only likely to take place during prolonged direct contact between animals.

It is not exactly known by what route and/or mechamism the virus infects the host. It somehow manages to escape the primary defence mechamism and establishes a persistent infection. Infection through the oral route by administering bovine colostrum containing cell-free OPPV to newborn lambs was described (101). This experiment did not permit conclusions as to whether

infection took place through the mucosa of the nasopharynx, the tonsils or lower down the digestive tract. Furthermore, the authors did not state the method of administration, but feeding with a bottle and teat may produce aerosols. Aerosols produced in the nasopharynx may reach the lung which is probably not the case with aerosols inhaled through the nose.

The mode of infection in newborn lambs may be different from adult sheep. Newborn lambs may become infected shortly after birth by contact transmission or possibly through ingestion of colostrum and milk (see next section). The exact route by which the virus infects the newborn lamb in case of lactogenic transmission is not known but two basically distinct routes can be suggested, (i) virus may pass the mucosa of the nasopharynx or lower parts of the digestive tract, primarily infect the tonsils or reach the lung in an aerosol, and (ii) monocytes/ macrophages are probably able to pass through the intestinal wall of the neonate and if such cells from the colostrum contain the provirus they act as a "Trojan horse". The role of the antibodies in the colostrum/milk is as yet undecided, but the option of cell-associated (pro)virus transmission offers an elegant way of escaping such antibodies.

Field epidemiology

Two different routes of transmission should be considered in OLV, (i) horizontal transmission probably mainly aerogenic and, (ii) vertical transmission, comprising genetic (trans-gametal through integrated viral genome), transplacental and lactogenic transmission.

Horizontal transmission

Most of the current knowledge concerning the epidemiology of OLV-infections is based on field observations. The occurrence of horizontal transmission was most dramatically demonstrated in Iceland where, approximately six years after the introduction of 20 imported Karakul sheep, the disease appeared in flocks where two of the rams had been introduced (15). As a result of a rather unusual husbandry conditions in Iceland, i.e. a long winter housing period and communal grazing, the infection spread rapidly in primary infected flocks and from these more slowly to others. The practice of autumn roundups where thousands of sheep from different flocks and districts are gathered in colleting pens for 1-3 days to be sorted out, was undoubtedly important in the secondary spread of infection (15).

In areas with a more moderate climate, and thus with shorter winter housing periods, horizontal transmission is likely to be less efficient and therefore infection will spread more slowly. If communal grazing in conjunction with round-ups is not practiced, flock-to-flock transmission will usually only occur through movement of infected breeding sheep.

With respect to transmission within a flock, it is important to note that the efficiency of horizontal transmission of OLV is strongly associated with husbandry conditions and management. Husbandry conditions are generally defined by the climate, i.e. necessity and eventual length of winter housing, and the intensity of production, i.e. stocking rate. Management deals with aspects of the handling of the flock under the given husbandry conditions e.g. breeding and culling strategy, parasite control, lambing system, handling procedures etc. Both husbandry and management define the conditions for, and thus the rate of, horizontal transmission. A possible genetic predisposition to infection and development of disease will influence the rapidity of spread and the incidence of disease, but probably not the epidemiological pattern as such. However, with the probability that animals with OLV-associated lung and/or udder lesions excrete more virus than unaffected (infected) animals, the presence of affected animals will further accelerate the spread of OLV in the flock.

As stipulated above, horizontal transmission occurs, but its degree depends on the husbandry and managements conditions of the flock together with a number of interrelated, yet undecided, factors. In moderate to warm climates, winter housing is usually short, or even unnecessary. In colder areas, however, winter housing, sometimes for periods of several months, and indoor-lambing are required and in this situation particularly the climate conditions in the shed can influence the degree of transmission. Although there are no hard data concerning the influence of the indoor-climate, it seems most likely that a moist, poorly ventilated shed offers ideal conditions for aerogenic transmission. The rapid spread in the Icelandic flocks (15) is an appropriate example in this respect.

Management can also exert an effect on the degree of horizontal transmission. the breeding strategy, for example, can contribute to the spread in a flock by unknowingly selecting sheep which happen to be infected. This will especially happen in the case where infected breeding sheep were introduced. Their number will usually increase, and with it the number of in-

fected sheep, because the progeny of infected ewes have a considerable chance of being infected was well (see below). At the same time the chance of horizontal transmission will also increase. A similar effect will obviously be obtained when favoured lines of breeding happen to be infected. Of course, the opposite effect is obtained when uninfected lines are selected. Another, probably less dramatic example of the effect of management on the degree of transmission is an inadequate culling strategy. If affected ewes and their progeny are not culled, a higher chance of further spread will be the result. A clear example of considerable horizontal transmission amplified by particular selection was described by Houwers and Van der Molen (84) in their long-term study of natural transmission. Five years after introduction of two infected ewes, 83 per cent of the flock appeared to be infected.

From the point of view of possibly increased virus excretion in affected sheep, early culling of such sheep will limit further horizontal transmission.

A last example of managemental effects on the spread of infection is feeding lambs colostrum from other ewes or pooled colostrum. If one or more infected ewes are accidentally used as donors, it is likely that the virus will be transmitted to the lambs as lactogenic transmission is probably relatively efficient (see below).

The epidemiological role of rams within flocks is not important, because there are no indication of the virus being specifically transmitted with semen (87) or during mating. However, rams are incriminated in the transmission of the infection between flocks because they are especially moved between flocks. Most flocks introduce rams annually. When an infected ram is introduced, the chances of horizontal transmission depend on the husbandry situation and the management as in the case of introduction of an infected ewe. the only difference is that the progeny of the ram have no extra chance of being infected whereas the progeny of an infected ewe are particularly at risk. If the ram(s) are removed from the flock before the winter housing period, the risk of horizontal transmission is usually low. Nevertheless, the fact that the introduction of rams is an almost annual event in every flock means that they have contributes considerably to the spread of OLV between flocks and sometimes even between populations.

Insect and parasite vectors of the virus have not been described, and so far no epidemiological event has suggested a possible role of a vector. Consequently, little work concerning this subject has been done. The sheep ked (Melophagus ovis) is probably one of the major candidates and equivocal results have been obtained with this parasite (102). Its life-cycle generally takes place on one host and therefore it can hardly be expected to contribute significantly to horizontal spread of the virus. As yet, no indications have been obtained for other blood sucking insects to act as vectors in moderate to cold climatic areas. The situation in (sub)tropical areas is still unclear. The possible role of the small lungworm of sheep (Muellerius capillaris) was investigated and no evidence for transmission of MVV with the larvae was obtained (103).

Cases of iatrogenic transmission have so far not been recorded. However, the possibility of such an event cannot be excluded. Needle-transmission during vaccination procedures is not likely to be significant as the virus is mainly monocyte/macrophage-associated and the number of (pro-) virus carrying monocytes in the blood is extremely low (91, 95). Moreover, most ovine vaccines are administered subcutaneously and this will reduce even further the chance of transferring infected cells. Experiments in this respect were negative (87). Blood sampling procedures using one needle may, however, exert a larger, but still low, risk of transmission. Administration of drenches with a special drench-gun may also introduce a low risk of iatrogenic transmission as lacerations of the throat can be caused.

In conclusion, iatrogenic transmissions can occur but seem unlikely yo contribute significantly to the epidemiological pattern.

Vertical transmission

Genetic and transplacental transmission: De Boer (19, 104) and Gudnadóttir (105) found no indication of these types of transmission in their attampt to isolate MVV from fetuses derived from infected ewes. As part of a larger study, absence of infection was demonstrated in a group of approximately 40 lambs which were separated from their infected dams immediately after birth and reared in isolation, followed by serological monitoring for several years and subsequent pathological and virological examinations (103). Similar results were reported for OPPV by Light et al. (106). Further evidence in this respect was provided by Houwers et al. (107) in their

study on the feasibility of artificial rearing of colostrum-deprived lambs. On the other hand, Cross et al. (77) assumes vertical transmission in OPP -affected ewes on histological grounds. Cutlip et al. (108) managed to isolate OPPV from 1 fetus and 2 newborn lambs out of a total of 70 fetuses and lambs examined. The recorded difference between MVV and OPPV as regards genetic and/or transplacental infection might be accidental, but could also reflect strain differences. These investigations did not distinguish between genetic and transplacental transmission, although the latter seems the most likely option.

However, sufficient evidence is available to conclude that genetic and/or transplacental transmission only occurs as an exception. Consequently, it is concluded that lambs from infected ewes are generally born uninfected.

Lactogenic and/or ewe-to-lamb transmission: Lactogenic transmission has not yet been proven to occur, but several observations suggest that it does. The virus has been isolated from the colostrum and milk of infected ewes on several occasions (19, 109). De Boer et al. (103) showed that infection can occur within 10 hours after birth, and they suggested the possibility of lactogenic transmission although they could not exclude the nursing of the lamb by the ewe (contact transmission) as another possibility. This study also showed that the efficiency of transmission, be it lactogenic or/and by contact, is clearly associated with the duration of exposure, i.e. lambs left with their dams for 10 hours proved to be infected to a much lesser degree than those which were left with their dams for a period of six weeks.

Different results in this respect were reported by Straub (110) who left 16 lambs with their dams (infection rate and age not stated) until two months of age and found no serological evidence of infection during the following observation period of two years. These limited results suggest a low efficiency of lactogenic and/or ewe-to-lamb transmission.

On the other hand, several serological studies gave strong indications of seropositive ewes infecting their progeny in flocks with a low incidence of infection (111). In addition, a retrospective analysis of 214 ewe-progeny relationships in an experimental breeding flock with a 37.9 per cent seroprevalence showed that progeny of seropositive ewes were twice as likely to be seropositive than progeny of seronegative ewes (88). For one-year-old progeny this was even more than three times as likely. These results

demonstrate the epidemiological importance of the ewe-progeny relationship. In flocks with moderate to high levels of infection such associations are largely masked by the degree of horizontal transmission.

For the caprine lentivirus (CAEV), however, Adam et al. (112) and Ellis et al. (113) provided unequivocal evidence for lactogenic transmission by transferring the infection with colostrum and milk to kids. In addition, Oliver et al. (114) demonstrated transfer of CAEV with colostrum/milk from infected goats to lambs. From these results lactogenic transmission of CAEV appeared rather efficient. In view of the far-reaching biological, pathological and epidemiological similarities between CAEV and OLV it is most likely that the resemblance extends to lactogenic transmission as well.

Although conclusive evidence is still lacking, it is felt that lactogenic transmission is an important feature of the epidemiology of OLV. However, ewe-to-lamb (contact) transmission may be important, too.

With rising levels of infection in a flock, horizontal transmission becomes increasingly important in terms of its relative contribution to the total spread, while the contribution of lactogenic or ewe-to-lamb (contact) transmission will remain steady as a consequence of its simple one-sided character.

CONTROL OF OLV

Prevention

The prospects for development of a conventional vaccine are rather bleak due to the peculiar character of this virus infection. The intensive research currently performed with respect to the human lentivirus HIV may result in a basically new approach which could impose prospects for an OLV-vaccine. However, even if an OLV-vaccine becomes technically feasible in the future, the economic aspects of its application will probably be questionable. Consequently, the only options available now, and most probably during the next five to ten years, are preventive measures and control (eradication) programs.

Populations still free of OLV should be protected from infection by preventive measures. On a national level, this can be achieved by implementing adequate import conditions. Such conditions should be based on certification of freedom from OLV of the flocks of origin. Such certification can only be provided by recent serological examination of the whole flock

of origin, with negative results. If the sheep population of the country of origin is known to be infected, two flock tests with a minimum interval of six months, including all animals over 12 months of age, should be a minimum requirement. Further conditions should cover other aspects, e.g. additions to the flock of origin, but these are usually incorporated in the general import conditions.

In addition to control on a national level, the individual flock-owner can also take action with respect to prevention, e.g. by purchasing breeding sheep or production stock from known healthy flocks, instead of buying through the market. A next step could be requiring information on the serological status of the supplying flock. Once preventive measures on a flock basis get to this stage, the need for a guided and controlled approach, i.e. an official program, in clearly felt. In the next section the basic ideas underlying such a program will be discussed as well as its feasibility and efficacy.

Basis of control at national level

In countries with relatively intensive sheep production, different sectors can commonly be distinguished within the production cycle. The pure breeding sector is engaged in producing sheep of a perticular breed. The second sector mainly produces production ewes (usually crossbreds) for the third sector, the (fat or finished) lamb-producing flocks ("commercial flocks"), which form the last step in the production cycle. The different sectors are usually associated with particular geographical circumstances and many farms are active in more than one of the three sectors.

In addition, because of the varying geographical circumstances, many areas have developed a typical, mostly uni-directional, flow of production and breeding sheep, so-called stratification, e.g. sheep used for breeding on the hills are sold to flocks on lower land to be used for crossbreeding when they have reached a certain age. Both sector-associated production and stratification determine the general flow of breeding and production sheep, i.e. sheep not destined for slaughter. In areas where wool is the main product, particular patterns of flow of sheep may also exist.

In OLV-endemic areas, control should concentrate on establishing a program which can provide OLV-free breeding and production sheep. Once the buyers become aware of the advantage of OLV-free sheep, a demand will develop encouraging flock owners to provide such a product. Meanwhile, the

flock owners who cannot supply the demand in the market will find a decreasing interest in their product and will thus eventually be forced to take part in the program. In fact, a voluntary control program ultimately depends on, and at the same time exploits, the free market mechenism. Ideally, this approach should be used for OLV in conjunction with other infectious diseases, e.g. chlamydial abortion, to further increase its impact.

The basis of the program is serological examinations to identify infected animals. The availability of sensitive, reliable and labour-efficient serological techniques which yield similar results in different laboratories is of paramount importance. The techniques hitherto used all have advantages and disadvantages and require expertise to yield unequivocal results. A recently developed improved ELISA employing monoclonal antibodies in a blocking principle appears to be the most promising so far (115).

As outlined in the chapter on diagnostic methods, a negative serological test of an individual sheep does not yield reliable information. A negative test of a whole flock affords relatively reliable information as to the status of the individual sheep and this will further increase with repeated negative tests of the flock.

Experience with the Dutch MV-control program indicates that two successive negative flock tests with a minimum interval of six months yield a sufficient level of reliability (116). More flock tests will marginally improve this, but requirement of more than two flock tests reduces the practicability.

Besides serological examinations, a set of regulations should be imposed to control the risk of (re-)infection of the flock, e.g. by introduction of infected sheep or direct contact with infected sheep (see next section).

The lamb production sector comprises the main part of the sheep population and sells its product for slaughter. Consequently, an OLV-free status does not add value to its product, but economic damage resulting from OLV-infection should be prevented. This is not to be achieved in terms of active control (participation in a control program) but by passive control, basically by taking the opportunity to obtain OLV-free offered by both other sectors ("control by management"). When most breeding and production stock is cleared of infection (first stage), further spread is virtually under control, and a sufficient number of OLV-free flocks will be available to gradually replace infected production stock (second stage).

In the lamb production sector, the market does not require freedom from OLV and thus awareness in this respect will generally be low. As the financial damage is not easily recognized, because of its hidden and complex nature, it is particularly crucial to stimulate awareness and to motivate action. After all, the ultimate goal of efforts to control OLV lies in prevention of damage to this sector.

Complete eradication of OLV from a defined population is theoretically feasible if a rigorous compulsory test and slaughter program is continued long enough. The practical situation in many countries may, however, not be suitable for complete eradication and thus other, less demanding and more praticable, approaches should be sought, e.g. a voluntary control program.

Examples of national OLV-control programs

Programs to control OLV are presently underway in a few countries. The general design is adapted to the national situation on the basis of the initial incidence of infection and its pattern of spread. When implementation of a control program is considered, the first information required is the incidence of infection and the pattern of spread in conjunction with the structure of the production cycle, and any existing stratification pattern, i.e. the general flow of sheep. Secondly, the structure of the sheep production associated organisations, e.g. breeding societies, and the structure of animal health care should be considered. The design as well as the execution of an OLV-control program requires communications between, and the participation of, the many organisations and structures which are usually involved with sheep production.

Norway for example made OLV notifiable in 1973 and imposed restrictions of flocks which proved to be infected at serological examination and/or slaughterhouse inspection. Such flocks were only allowed to sell for slaughter. The flocks tested contained imported or progeny of imported Texel sheep, or had contact with flocks containing such sheep. Over the years some 135 flocks were found to be infected and, as a result of the restrictions imposed upon them, most have been slaughtered. This approach appears to be effective as the number of infected flocks detected annually has now almost reached zero. Meanwhile, over 200,000 serum samples were tested (117).

In Denmark, some 30% of the flocks and 11% of the animals initially appeared to be infected, indicating a relatively low incidence of infection. In 1979, a voluntary control program was initiated based on repeated sero-

logical testing associated with several degrees of freedom and a final "free" stage wherein the flock is retested annually for two years and thereafter at three-year intervals. As the initial incidence of infection was generally low, the reactor-free status was easily reached by culling of seropositives and their progeny. At present, an estimated 800 flocks have achieved status "free", the average flock size being approximately 10 (118).

In the U.K., OLV was apparently recently imported with sheep from the European continent (32) and thus the infection appeared to be mainly limited to flocks containing exotic breeds. However, infection was recently also reported in flocks consisting solely of indigenous breeds and crossbreeds without traceable contacts with known infected flocks, indicating a greater penetration of the sheep population than hitherto expected (119). Still, the incidence of infection in the total sheep population is probably very low. A voluntary flock-accreditation scheme was launched in 1982 based on repeated flock testing with an interval of six to nine months, the number of "clear" tests required for accreditation depending on whether the flock consisted solely of indigenous breeds (2 tests) or contained imported sheep, their progeny or contacts (3 tests). Expenses were partly covered by the government. Seropositive sheep were slaughtered and no compensation was paid. An annual "clear" flock test is required to maintain accreditation. At present, over 2500 flocks have obtained accreditation. From April 1987 the scheme is changed in a few aspects, i.e. the procedure will completely be at the owners' expense, some regulations will be simplified and the total number of sheep to be tested in the different stages will be decreased by increasing the minimum ages of the eligible animals and by introducing statistical sampling (120).

The initial situation in the Netherlands was rather different from that in the countries hitherto mentioned, as a limited amount of testing suggested that the incidence of OLV was comparatively high. Thus 80% of 151 larger flocks (> 50 ewes) tested appeared to be infected (21) and individual flocks with an incidence up to 70% or more were not unusual. In 1982 a voluntary control program was launched based on accreditation of flocks which had passed two consecutive tests with an interval of six months. All animals over six months of age were tested. Accreditation had to be renewed every 12 months by passing a flock test comprising all sheep over 12 months of age. If seropositives were found at any test, the positives plus progeny were removed and the flock had to start again at the beginning. In

addition, the owner had to follow strict rules to avoid contact with non-accredited sheep and only accredited sheep (including rams) were allowed to be added to the flock. The restriction on the addition of rams in particular made many flock owners unwilling to participate. The whole operation was at the owners' expense which also added to their reluctance. Nevertheless, the number of participants rose quickly, mainly as a result of the demand in the market and by the end of 1986 over 1800 flocks (mean flock size 20 ewes) were participating, which is 80 per cent of the registered breeding flocks. Separate shows and competitions for accredited stock were organized and today such events for non-accredited sheep are virtually non-existent; the breeding societies will soon be "accredited only".

After four years of experience with the program it was decided that some regulations could safely be relaxed. Twelve months after obtaining accreditation the flock has to pass a test as usual and thereafter testing will be required every 24 months. If a flock test is failed and accreditation withdrawn, one test six months after the removal of the seropositived and their progeny is sufficient to regain accreditation. In view of the high incidence of infection in the sheep population, the Dutch sheep breeders have obviously invested a lot of energy and money to get this far. The methods used will be discussed in the next section. A detailed report on the first four years of the Dutch OLV-control program is given elsewhere (116).

These examples show that different approaches, adjusted to the particular circumstances, can yield good results. It should be emphasized that in view of the particular nature of this infection, especially the existence of a provirus in conjunction with sometimes very late or no seroconversion, an absolute guarantee as regards freedom of OLV cannot be provided, not even in accredited flocks. However, the risk of accredited sheep carrying the virus is low (116).

Methods of OLV-control at flock level

Total replacement

Total replacement of an infected flock by OLV-free stock is a rather drastic approach, but gives an immediate result and may be attractive from an economic point of view (76). However, in the case of a breeding flock this approach means the loss of valuable genetic material.

Repeated testing and culling of seropositives

The feasibility of half-yearly testing and culling as a method of control at flock level was initially shown in experimental flocks (121, 122) and confirmed in a field trial with 15 flocks among which three initially had a seroprevalence of over 50 per cent (123). The results of the latter study suggested that culling of the seropositives plus all their progeny, including those of preceding years, would accelerate the process. In theory, this was to be expected as the progeny of infected ewes have an increased chance of being infected (88, and later section). The culling of all progeny should thus be regarded as a precautionary measure. Sucking lambs of seropositive ewes should be culled in any case.

Repeated serological testing and culling of seropositives is especially recommended for flocks with a low incidence of infection, i.e. < 30 per cent, otherwise the size of the flock may decline to an economically unacceptable number. An initial reduction in size can, however, be compensated for by purchasing OLV-free stock. With half-yearly testing of all sheep over 12 months of age, the number of seropositives will rapidly decline and zero per cent will usually be reached within three to five tests.

If a higher incidence of infection is present and the farm situation permits running of two separate flocks, the reduction of flock size can be overcome by running separate seropositive and seronegative flocks after the first test. The positives detected in the "negative" flock at subsequent tests are transferred to the infected flock. The negative flock will increase in size while the infected flock decreases in size until it can entirely be replaced.

Accurate flock recording in conjunction with proper identification of individual sheep is obviously essential when repeated testing and culling is chosen as approach. The most reliable identification is application of ear tattoo number.

Artificial rearing of ovine colostrum-deprived lambs

The basic principle of artificial rearing of ovine colostrum-deprived lambs and its feasibility under laboratory conditions were demonstrated as part of an epidemiological study initiated in 1967 (19, 103). Similar results, also under laboratory conditions, were obtained by others (106). The feasibility of this approach under field conditions was shown by Houwers et al. (107) in a study comprising 11 flocks, where 389 lambs were separated

from their dams immediately after birth, fed bovine colostrum and reared on artificial ovine milk replacer. Comparative studies of some blood and serological parameters indicated that bovine colostrum could serve as a useful alternative to ovine colostrum (124, 125).

Within the framework of the national OLV-control program in the Netherlands, over 5,000 lambs have been reared in this way in 185 flocks. Eventually, 1.1 per cent of these lambs in 23 flocks seroconverted (116). These results indicate that the average sheep farmer working under variable farm conditions is able to perform the procedure with reasonable success. Specific veterinary problems have not been encountered other than an anemia caused by a factor present in the colostrum of some donor cows (126). A relativelt simple laboratory technique was developed to detect this factor in batches of bovine colostrum and the subsequent exclusion of positive batches was shown to be an effective preventive measure (127).

With respect to the procedure of artificial rearing the following general recommendations can be given:

- Following attended birth the (ewe-)lambs must immediately be separated from their dams. They must be transferred to a separate room, or if this is not available, to a separated section of the lambing shed.
- During the first 24 hours the lambs must be bottle-fed 200 to 400 ml bovine colostrum at body temperature from a stock kept at -20°C.
- Lambs of the same age must be penned in groups of a maximum of 10 to 15 animals and accustomed to a lambs bar containing ad libitum artificial ovine milk replacer at room temperature. One teat should be available for every two lambs. Hay and concentrates should be available from 14 days onwards.
- Lambs should be vaccinated with an appropriate clostridial vaccine at two weeks of age and before weaning.
- Weaning at six to seven weeks of age can be achieved by abruptly replacing the milk with water.
- After turning out to pasture caution must be taken as regards coccidial infections and if necessary, concentrates premedicated with a coccidiostat can be fed intermittantly.
- Separation from infected animals should be maintained as much as possible under the given circumstances. Experience has shown a spatial separation

of two meters to be sufficient in most cases.

- Ewes that have no lamb to rear should be put on a poor ration/pasture to accelerate drying off. The udder should not be touched.

Serological examination of the lambs should be performed repeatedly at intervals of six months starting at an age of six months. A few seropositives should not be regarded as an indication of complete failure of the procedure because experience has shown that, if positives do appear, their number will usually remain small (116). Nevertheless, such lambs should be removed as soon as possible to limit the chance of horizontal spread.

It is important to note that a different view with respect to the efficiency of ewe-lamb transmission has been presented (110). Preliminary results from field experiments in West Germany suggest that a less stringent approach to artificial rearing is possible without significant infection of the lambs, i.e. separation from the dams after two days and thus after ingestion of colostrum. However, further investigations are required to substantiate this view. Meanwhile, the prudent approach of separation immediately after birth is evidently the safest. A change in the present epidemiological concept along these lines would, nevertheless, considerably facilitate the possibilities of control.

Artificial rearing of colostrum-deprived lambs is a labour-intensive and generally expensive operation and should therefore only be applied when valuable genetic material has to be salvaged. Usually, two to three seasons of artificial rearing produce a sufficient number of ewe-lambs/ewes, even with moderate selection, to replace the parent flock. The use of a selected group of infected ewes as lamb donors will result in a slower replacement of the entire flock, but may have the advantage of simultaneous genetic improvement.

Annual purchase of OLV-free replacements

In the case where accreditation is not required, i.e. in lamb production flocks, OLV-infection can probably be controlled by annual purchase of all replacements from accredited flocks. This approach has not yet been evaluated in the field and is thus hypothetical. Horizontal spread of OLV will be relatively slow under most husbandry conditions and through annual addition of uninfected stock, the infection in the flock will be "diluted". As a result, the incidence of infection in the flock can be expected to decline, which in its turn causes a decline of horizontal spread. Theoretic-

ally, the incidence of infection could approach zero per cent in four to six years. If it is subsequently decided to entirely clear the flock of infection, testing and culling should be applied.

Although this approach has not yet been proven to be effective in the field, it might be useful, especially for lamb production flocks. Annual purchase of OLV-free (accredited) replacements could become the ultimate example of "OLV-control by management".

Alternative approaches

Alternative approaches to OLV-control are presently under study. Results obtained by Sihvonen (109) indicated a low degree of ewe-lamb transmission in early stages of infection, while ewes in a later stage of infection readily infected their lambs. In addition, observations of a Dutch shepherd and a retrospective analysis of the eventual seroconversion of daughters in association with the age of the mothers at the time of lambing (76) suggested that progeny of ewe-lambs are infected to a lesser degree than the progeny of older ewes. Possibilities for exploitation of this phenomenon were tested. In a severely infected flock the yearlings lambed separately and the ewe-lambs born were kept as a separate flock after weaning. A total of 43 per cent of the yearlings was seropositive. The new flock was repeatedly tested for several years and only 2 of the original 43 seroconverted (76). These results indeed suggest a less efficient transmission of the virus from yearling ewes to their progeny. However, it can be argued that the separation from the flock also contributed because of reduced exposure to horizontal transmission. Similar experimental approaches are in progress in a few other flocks.

In another experimental study, the ewe-lambs from seronegative ewes were selected after weaning to form separate flocks, which are subsequently tested at half-yearly intervals. The preliminary results indicate that this approach can be successful, too. These partly preliminary results indicate options for other methods of "control by management". However, considering their practical implications and the often restricted possibilities in the field together with the questionable economics, these methods will probably only be applicable in a limited number of flocks.

General conclusions

The choice of method of OLV-control in a given flock depends on a va-

riety of conditions. First, the objective of control is to be considered, i.e. accreditation (eradication) in the case of breeding/production-stock producing flocks or just control in lamb producing flocks. The availability of an already accredited population of sheep is a second important factor. Furthermore, the incidence of infection in the flock and the practical possibilities on the farm are crucial.

A cost-benefit analysis is hard to make, but it is important to realize that serological examinations (sampling and testing) are relatively expensive and should thus be limited as much as possible.

Total replacement or annual purchase of OLV-free (accredited) replacements are the most attractive approaches for lamb-producing flocks. If a flock contains valuable breeding stock and accreditation is required, repeated testing and culling or artifical rearing are options depending on the infection rate. Combining of different methods is obviously feasible, e.g. testing and culling or replacement of the bulk of the flock, in conjunction with artificial rearing of lambs from selected top-quality breeding ewes.

Finally, OLV-control should be considered a matter of long-term thinking, commercial attitude and common sense, and should be a joint effort between the sheep industry and animal health care organizations.

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7

PATHOLOGY AND EPIDEMIOLOGY OF LENTIVIRAL INFECTION OF GOATS

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ABSTRACT

Lentiviral infections occur in goats throughout the world, and infected animals remain infected for life. Unlike the lentiviruses of sheep, goat lentiviruses produce clinical signs in both kid goats and adults, but the clinical syndromes differ greatly between the two age groups. Leukoencephalomyelitis is the primary manifestation of disease in kid goats, but lentivirus infected adult goats typically have a chronic, progressive arthritis. In both age groups, subclinical disease is common. Chronic interstitial pneumonia and chronic mastitis are seen in infected goats as they are in the lentiviral infections of sheep. Immunosuppression of infected goats does not alter viral replication, and lesions do not occur in infected immunosuppressed goats. Lentiviral infections of goats and sheep share several pathological, as well as epidemiological similarities, but they differ primarily in that clinical expression of disease in goats occur in both young and adult animals.

INTRODUCTION

Lentiviral infection of goats occurs in North and South America, Great Britain, Europe, New Zealand, and Africa (1); following infection, goats remain persistently infected for life. The clinical expression of disease varies from region to region and from herd to herd (2). This disorder can affect both kid goats and adults, and it is recognized by various names: infectious (viral) leukoencephalitis of goats (3,4), or caprine-arthritis encephalitis. Granulomatous encephalitis of goats (5) also probably fits within this group, based on the common clinical and morphological features, although the etiological agent was not identified in the original report. In most

cases, the disease affects multiple systems, and subclinical infection/disease is the rule, rather than the exception. Serological survey of more than 1000 goats from 24 states within the United States showed that 81% were seropositive (6). Goats in Canada, France, Norway, and Switzerland also have a high incidence of infection (1). Limited prospective studies of naturally infected goats indicate that half or more of seropositive goats will develop clinical disease (6).

Although the goat lentiviral infections are "slow viral infections" in the sense that the time between infection and disease expression may be long (months to years), experimental studies show that lesions appear within days post-inoculation, despite the fact that clinical disease (particularly neurological disease) may not appear for many months. This review will summarize the clinical and pathological features of goat lentiviral infection, the pathogenesis, and factors that may contribute to the variation in disease expression seen in this infection.

In North America, two major clinical patterns of disease have been recognized -- encephalitis and arthritis -- the latter is usually associated with a wasting syndrome. Goat lentiviral infection affects multiple organs: brain and spinal cord, joints and other connective tissues, lungs and mammary glands. Inflammatory lesions are characterized by a lymphoproliferative response. Lesions are immunologically mediated; immunosuppressed, virus-infected goats do not develop lesions (7).

Central Nervous System

Clinical Disease. Neurologic disease is most often seen in kid goats, 2-6 months of age, but also occurs in adult goats. Kid goats develop weakness, ataxia, and/or paralysis. They are usually afebrile, bright and alert and will continue to eat and drink when assisted. Their cerebrospinal fluid may show a pleocytosis. Neurological signs may progress so that the animal dies or must be euthanized, however, some goats have relatively mild impairments and may live for several years. Neurological disease in adult goats can follow a similar pattern, more often, they have a sudden onset of neurological signs associated with fever, depression, blindness, and

other signs typical of a meningoencephalitis. Occasional animals show mild neurological deficits for years without evidence of progression.

Pathological Changes. Lesions within brain and spinal cord, visible grossly as tan or rosy-pink discolorations of white matter, are multifocal, perivenous, and typically located in white matter of the cerebellar peduncle or in subpial, subependymal, or periventricular regions. In the spinal cord, lesions may expand outward from a central canal containing cellular debris; alternatively, lesions may extend inward into the white matter along penetrating vessels from the leptomeninges. Lesions may be quite limited, involving only one segment of spinal cord, or they may be distributed diffusely throughout the neuraxis as focal or multicentric coalescing nodules.

Lesions vary greatly in severity. The mildest and earliest lesion (within less than one week following experimental infection) is a perivascular accumulation of lymphocytes and microglia within white matter, usually in the subependymal region. Lesions are circular or nodular with an ill-defined center. Within a few days to a week, this initial focus expands along the course of the vessel and extends outward into the neuropil; inflammatory cells comprising the host's response are a characteristic mixture of macrophages, monocytes, lymphocytes, and plasma cells. The disporportionate numbers of macrophages, the target cells for lentiviral infection (8) are the result of increased division at the site of the lesion (9). Syncytial cells, epitheloid or giant cells are rare. The character of the inflammatory response remains the same for many weeks. Neutrophils occur in very necrotizing lesions but are an unusual feature. Primary demyelination (loss of myelin sheaths without loss of axons) is typical, but axons may also be destroyed in severe lesions.

Kid goats that survive the initial phase of neurological disease have persisting lesions within the CNS. Areas of primary demyelination with some evidence of remyelination, gliosis, and increased connective tissue around vessels occur in the same distribution as that described previously. Some inflammatory infiltrate persists, even after many years, but it is relatively mild in burned-out

lesions and consists primarily of lymphocytes.

The severity and extent of lesions in the nervous system varies from animal to animal. In general, severe lesions are more common in kid goats - lesions may be so destructive that there is a transverse myelitis, and the cord appears infarcted. Because the topography and severity of lesions determine clinical signs, kid goats with severe lesions of the spinal cord, cerebellar peduncles, or brainstem will have detectable clinical signs and are more likely to be diagnosed. Only 10-15% of kid goats infected at birth develop neurological disease (3). However, in infected herds, subclinical CNS disease (usually involving the white matter of the cerebral hemisphere) is the rule. Autopsies of more than 20 kid goats less than 6 months of age (only one of which had developed clinical disease) revealed typical lesions in all but one animal (10). Thus, in infected herds, many kid goats are infected at birth via colostrum from infected does, or possibly intrauterine infection (11), and have subclinical neurological disease throughout life. Many of these goats will subsequently develop arthritis.

Experimentally infected adult goats develop CNS lesions identical to those of similarly infected kid goats, and clinical neurological disease in adult goats may also occur in field situations. Clinically affected adult goats, like kid goats, develop necrotizing lesions in regions (e.g. spinal cord) that are likely to produce neurological signs (5). More often, chronically infected adult animals have bland, nodular lesions, consisting of small clusters of lymphocytes and macrophages, which are located in the white matter of the cerebral hemispheres, and these lesions rarely produce clinical signs. Thus, subclinical CNS disease is common in adult goats. More than 50% of adult goats with lentiviral arthritic disease will have subclinical CNS lesions (12,13).

Arthritic Disease

Clinical Features. The most common clinical manifestation of lentiviral infection, occurring in 25% of seropositive adult goats, is arthritis. Goats are usually 1-2 years of age when swelling of the carpus, and subsequently, the atlantal or supraspinous bursae are detected. Radiographic changes are initially those of soft tissue swelling, but, over time, this gives way to calcific deposits of

perarticular tissues, with mild periosteal and periarticular changes. As lesions progress, periarticular tissues become mineralized, and articular bones degenerate and collapse (13).

Lameness and associated decreased mobility of joints may progress insiduously, or the arthritic condition may be virtually static. Physiological stress, e.g. inclement weather, tends to exacerbate the disease. As the disease progresses, wasting occurs; the goat becomes emaciated with a shaggy, lusterless coat.

There is considerable individual variation in the course of disease. In a prospective study of naturally infected kid goats, almost half had well developed clinical disease by two years of age, although only one had advanced disease; by three years of age, half had severe disease (13). Despite the fact that the number of goats studied was necessarily small, the pattern is consistent with observations in the field.

Pathologic Changes. Experimentally, lesions appear 7-9 days following infection as focal necrosis of synovial lining cells and edema (14,15,16). In the subsequent week, synovial lining cells undergo hypertrophy and hyperplasia, collagen is disrupted and increasing numbers of inflammatory cells infiltrate the synovium. Fibrin deposits are intermixed among necrotic synovial cells on the hypertrophied villi. By a month post-infection, synovial villi are markedly hyperplastic and are infiltrated by large numbers of mononuclear cells, particularly plasma cells (often containing Russell bodies) and lymphocytes that tend to form germinal centers. Virus-like particles have been detected by electron microscopy in the three types of synovial lining cells between 18 and 45 days post-infection (16).

Within the first two months following infection, levels of intrasynovial IgG1 increase and peak at six months at 20 times normal levels. The increase in immunoglobulins occurs in concert with increased numbers of plasma cells and appears to be the result of intrasynovial synthesis (17). Immunoglobulins present within the joint appear to be directed in part against viral antigens (gp 90 and gp 125 (18).

The joint lesions, like the brain lesions, are immunologically mediated since virus-infected immunosuppressed goats do not develop

joint lesions, and goats immunized with inactivated virus and subsequently challenged develop severe, acute arthritis (7,19).

Pulmonary Disease

Although virtually all goats infected with goat lentivirus develop some pulmonary lesions, clinical disease is virtually unknown. This is in contrast to the situation in sheep where maedi and progressive pneumonia typically produce pulmonary signs of dyspnea (20). Interstitial pneumonia occurs in goats 7-10 days following experimental infection. Alveolar septa are infiltrated with mononuclear cells and peribronchial and perivascular lymphoid tissues become hyperplastic. In kid goats inoculated intra-bronchially by aerosol, hyperplasia of the lymphoid germinal centers is especially prominent (21). Occasional goats, usually debilitated by CNS disease, will develop a secondary bacterial bronchopneumonia. In these animals, alveolar lining cells proliferate and many cells are sloughed into the lumenae. Chronically infected goats have mild pulmonary lesions, but there may be increased connective tissue in alveolar septae. Lymphoid germinal centers may be so large as to be visible grossly.

Mammary Lesions

Mammary lesions have been recognized in infected adult does as infiltrations of mononuclear cells between the acini. In some regions, there is an apparent increase in connective tissue. Lymphocytes accumulate in nodules and form germinal centers around, and protruding into ducts, features that are of more significance with regard to transmission of disease since this location allows ready egress of infected leukocytes into milk or colostrum (14).

CONCLUSIONS

Lentiviruses of goats and sheep are closely related agents that produce persistent infection and neurological, pulmonary and arthritic disease (Table 1). Reasons for the differences in expression of clinical disease are not immediately apparent although different strains of the goat and sheep lentiviruses may be better adapted to replication in one tissue than another. Particularly intriguing is the effect of age on the incidence of neurological disease in goats. Several factors may play a role in this

Table 1. Comparison of Goat and Sheep Lentiviruses

	Caprine Arthritis Encephalitis	Maedi	Visna	Progressive Pneumonia
Clinical Disease				
Young Animals	++++	-	-	-
Adults	+++	++++	++++	++++
Distribution of Lesions				
CNS:				
Young Animals	++++*	-	-	-
Adults	++**	+	++++	+
Lung:				
Young Animals	++++	-	-	-
Adults	++	++++	+	++++
Joints:				
Young Animals	++	-	-	-
Adults	++++	-	-	++
Mammary:				
Young Animals	-	-	-	-
Adults	++++	-	-	++++

* Lesions in kid goats are distributed throughout CNS, but frequently involve spinal cord.

** Lesions in adults are often in frontal white matter; in goats with clinical signs they typically involve spinal cord and brainstem.

phenomenon. Dairy goats have evidence of infection in the mammary gland (14) allowing for the ready transmission of infected leukocytes into colostrum and/or milk. Goat dairy management practices also foster the spread of virus between kid goats, because colostrum and milk are pooled and bottle-fed to kid goats, a practice that could facilitate the ingestion of large amounts of infectious leukocytes by many animals in a herd. It is also possible that the blood-brain barrier is less well developed in the neonate and that traffic of leukocytes into the brain may differ in neonates, either of which might permit entrance of larger numbers of infected cells into the nervous system.

Clinical arthritis is also more common in goats than in sheep, although arthritis occurs in some sheep (22). Goats, in contrast, have a greater resistance to clinical pulmonary disease than sheep, despite the fact that pulmonary lesions occur in both. It must be acknowledged that the severity of pulmonary lesions in goats appears to be less than in sheep.

Some investigators have had success in experimentally transmitting sheep and goat lentiviruses across species (23,24) although others have not (25). These differing results may represent strain differences in the respective agents, but they also point out the similarities in lesions found in lentiviral infections of sheep and goats under selected experimental conditions. Thus, the goat and sheep lentiviruses differ primarily in the age-incidence and clinical course of the disease as seen in the field but are quite similar in the pathogenesis of lesions in experimental circumstances.

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Variations in Clinical Disease During Replication of Lentiviruses

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INTRODUCTION

Nonsuppurative arthritis in goats and chronic dyspnea (maedi) and paralysis with wasting (visna) in sheep are slowly progressive diseases that are preceded by unusually long incubation periods and are caused by viruses that persist indefinitely and replicate at a slow restricted rate (1). Named to emphasize their slow pathogenic process, the agents have been designated "lentiviruses". The lentiviruses of sheep and goats are prototypes of a taxonomic group of viruses that have similar pathogenic processes in other species. The group includes the viruses that cause infectious anemia and immune complex disease in equidae (equine infectious anemia virus, EIAV) and lymphadenopathy and acquired immunodeficiency disease in macaques and humans (human immunodeficiency virus, HIV) (2).

The lentiviruses are a subgroup of the retrovirus family-enveloped, positive-stranded RNA viruses that replicate via a proviral DNA intermediate (3,4,5). Unlike the oncogenic members of the retrovirus family, the lentiviruses cause neither tumors in animals nor transformed cells in culture. Their host range is limited to single species and they infect cells of the immune system of these hosts. Virus replication in immune cells sets into motion a continuum of patho-physiological processes that on one hand restrict virus replication (causing "slow infection") and on the other, disrupt the immune mechanisms that regulate proliferation of different lymphocyte populations (6,7).

This sets the stage for immunopathologic disease. In ruminants this is manifest as proliferation of mononuclear cells (inflammation) (1), in equidae as proliferation of antibody producing B lymphocytes (immune-complex disease) (8,9) and in primates as diminution of helper T lymphocytes and neural infection leading to dementia (10,11).

The insidious onset and protracted course of the ruminant lentivirus disease are thought to be determined by the continuous production of small amounts of viral protein(s) during the slow persistent replication of the agent in the animal host. This type of virus replication is unusual among RNA viruses and the molecular events in the virus life cycle that regulate production of these proteins are poorly understood. In this chapter we will briefly review the basic molecular mechanisms of replication of retroviruses and extend these to the more complex life cycle of the lentiviruses. Further, using maedi-visna virus of sheep and caprine arthritis-encephalitis virus (CAEV) of goats as prototypes of the lentiviruses, we show that these viruses have wide differences in biological properties and in molecular mechanisms of replication in cell cultures. However, many of these properties are shared and the importance of these in pathogenesis of the disease caused by the viruses is highlighted in this chapter.

Disease in infected ruminants is referable to chronic inflammatory lesions in different organ systems that include the central nervous system, lungs, joints and the mammary gland. In sheep the main form of disease expression is dyspnea with more rare occurrences of arthritis and mastitis (1). A high prevalence of chronic neurological disease (visna) (12,13) complicating the dyspneic disease (maedi) occurred during a major epizootic of the latter disease in Iceland in the 1950's. However, CNS disease is relatively rare among non-Icelandic breeds of sheep. Multiple strains of viruses have been isolated from sheep in Iceland

(maedi/visna virus) and from sheep in other parts of the world including the viruses causing progressive pneumonia in sheep (the equivalent of maedi) in the United States and Europe. Caprine arthritis-encephalitis was first recognized as an infectious disease complex in 1974 (14). It is characterized by arthritis (3) and mastitis (15) in adult dairy goats and a rapidly progressive paralytic disease in newborn goats (14,16). Multiple viruses have been isolated from these animals in several countries.

Biological properties of the lentiviruses

The ruminant lentiviruses replicate productively only in cells of the natural host and in these, only certain cell types are permissive for replication. These include primary cultures of macrophages, a cell type that support replication of all the viruses (17). However, the efficiency of virus replication in cultured macrophages varies among different field strains of virus and also from culture to culture, depending on the donor animal. Moreover, infected macrophages may or may not develop cytopathic effects (CPE) and this is seen as a non-specific degeneration of the cells. Cell lines (GSM) derived from the synovial membrane of newborn goats are also uniformly permissive for replication of these viruses (18). GSM cells are much superior to macrophages as indicators of virus replication. The cells can be subcultured and cell progeny maintain their ability to replicate virus after several subcultivations. Further, the viruses replicate to higher titers in these cells than in macrophages and cause CPE characterized by multinucleated giant cell formation (syncytia or fusion).

Different strains of viruses vary in their virulence for GSM cultures. For example, Icelandic visna virus replicates rapidly in these cultures, causing fusion within 24 hours after inoculation at a multiplicity of 2 to 5 and lysis during the following 3 to 4 days. In contrast, studies on

replication of several strains of CAEV show a slightly slower rate of replication after inoculation at the same MOI as visna virus. CAEV inoculated cultures develop typical fusion CPE but this progresses at a slow enough rate that uninfected cells divide and replenish new host cells for virus replication. These cultures thus become persistently and productively infected (18). Different strains of field viruses from sheep or goats may follow one or the other pathways of replication, the type of replication being characteristic for each strain. Thus, sheep or goat viruses may cause lytic or persistent infection in these cells regardless of host or origin (19,20).

Fibroblastic cell lines derived from the choroid plexus of sheep (SCP) have been used routinely for propagation of Icelandic visna virus (21). This virus causes fusion and lysis in these cells, similar to the CPE it causes in GSM cells. However, many strains of progressive pneumonia virus obtained from sheep in the US and several strains of CAEV (more than 20) isolated in our laboratory from naturally infected goats replicate poorly in SCP cultures. SCP fibroblasts thus provide a novel *in vitro* biological marker for a number of these lentiviruses. This marker is of further value because all of the viruses that cause CPE in SCP cells are highly virulent for GSM cultures. The molecular mechanisms associated with this lytic type of replication are described below.

In addition to differences among the viruses with respect to virulence for cultured host cells, the agents also vary in their ability to cause fusion "from without". As mentioned above, all strains of lentiviruses cause fusion during replication in GSM cell cultures. However, some of the viruses can cause fusion from without after inoculation of the cells at a MOI of > 10 (22). This type of fusion occurs rapidly, within 4 h and does not require virus replication because induction of fusion occurs long before

completion of a single cycle of replication (approximately 20 h) and because fusion can be caused by inactivated concentrated virus. Icelandic visna viruses and occasional strains of progressive pneumonia virus and CAEV can cause this type of fusion (19; ON, unpublished observations). However, most strains of PPV from sheep and nearly all strains of CAEV do not cause this type of CPE (23; ON, unpublished observations).

The ability to induce neutralizing antibodies during persistent infection in host animals provides another marker which distinguishes among the lentiviruses. All of these agents have neutralization epitopes in their envelope glycoproteins. However, only some of these viruses induce neutralizing antibodies. Visna virus is a relatively good inducer of such antibodies whereas PPV and CAE viruses are relatively poor inducers (24,25). These foregoing parameters provide evidence of a great deal of phenotypic heterogeneity among the ruminant lentiviruses. Despite these differences however there is a high degree of genetic homology and antigenic cross reactivity among viruses. Further, regardless of their differences during interaction in cell culture, these viruses have the common property of causing persistent infections and slowly progressive disease after long periods of incubation.

Genetic Organization of the Lentiviruses

Lentiviruses are a subgroup of the retrovirus family. These are positive-stranded RNA viruses. The viral genome contains three genes that encode the major structural proteins of the virus: the *env* gene that encodes the viral glycoproteins, the *gag* gene that codes for viral core proteins, and the *pol* gene that codes for the viral RNA dependent DNA polymerase (RTase). The genes are organized on the genome (5'-3') *gag-pol-env* (Fig. 1). Lentiviruses have a genetic organization similar to retroviruses with the

addition of open reading frames (ORF) (i.e. potential protein coding regions) between the pol and env genes. These ORFs Q and S code for proteins which play a role in regulating the molecular events of virus replication (see below) and are unique to the lentiviruses (26,27,28,29).

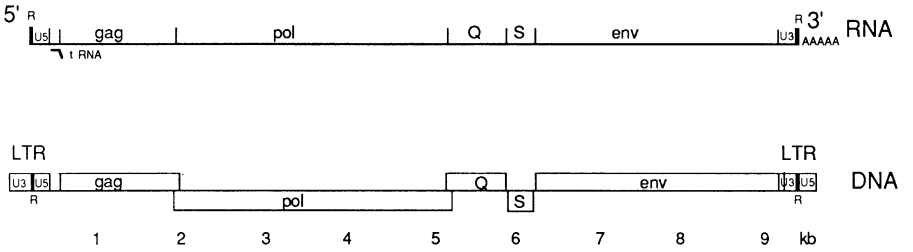


Fig. 1. Lentivirus genomic RNA present in virus and viral DNA present in infected cells

A major difference between the replication requirements of retroviruses and lentiviruses is the physiological state of the host cell. Retroviruses have a strong requirement for dividing cells which provide optimal conditions for DNA synthesis and integration of the proviral DNA. In contrast, lentiviruses replicate efficiently in non-dividing end-stage cells both in the host animal and in cell cultures. This allows for replication in the terminally differentiated macrophage populations and also for plaque formation in monolayer cultures of non-dividing cells. Since these viruses must utilize the cellular DNA polymerase to synthesize viral DNA similar to the retroviruses, they either provide additional viral-encoded replication functions or they activate the non-dividing cells to synthesize proteins and other factors that are required for DNA replication. The

effectors of these additional functions are probably encoded by the lentivirus genome and may be the products of any of three recently identified mRNA molecules whose functions at present are not known (30,31,32).

Reverse transcription of virus RNA into DNA

The genome of the lentivirus is considerably larger than that of retroviruses and is approximately 9.5 kb in length (28,33,34,35,36,37). However, similar to the retroviruses, the lentivirus genome has a series of unique non-coding sequences at each end, known as U5 and U3, in accordance with their location at the 5' and 3' ends of the RNA genome, respectively. At both ends of the RNA, flanking the U regions, are sequences which are identical to each other. These are the R (repeat) sequences. These terminal sequences on the viral RNA are important for transfer of all the genetic information from RNA to DNA as well as for creating structures at the ends of the viral DNA molecule which are important both for integration of the viral DNA into the host cell DNA and for the transcription of the proviral DNA back to RNA.

Each virus particle contains two copies of viral RNA and both copies are utilized for the reverse transcription of RNA into DNA. As the RTase copies the RNA into DNA, another enzyme activity of the RTase, RNase H, digests the RNA template. Reverse transcription begins at the U5 and proceeds for a short distance, going in a 3' to 5' direction, through U5 and R to the end of the RNA molecule (Fig. 2). The newly synthesized cDNA R region is complementary to the viral RNA and hybridizes with the 3' end of another viral RNA molecule. The RTase can "jump" to this second RNA molecule via the hybridized R sequences and cDNA synthesis continues through the U3 region, env, S, Q, pol, gag, U5 and R. For a second time the R region of the DNA hybridizes with yet another RNA molecule enabling the RTase to make a second jump

and synthesize the second copy of U3 sequences. The complementary strand of DNA is synthesized by cellular DNA polymerases resulting in a double-stranded linear molecule. (The molecular mechanisms of retrovirus replication are reviewed by Varmus and Swanstrom) (38,38a).

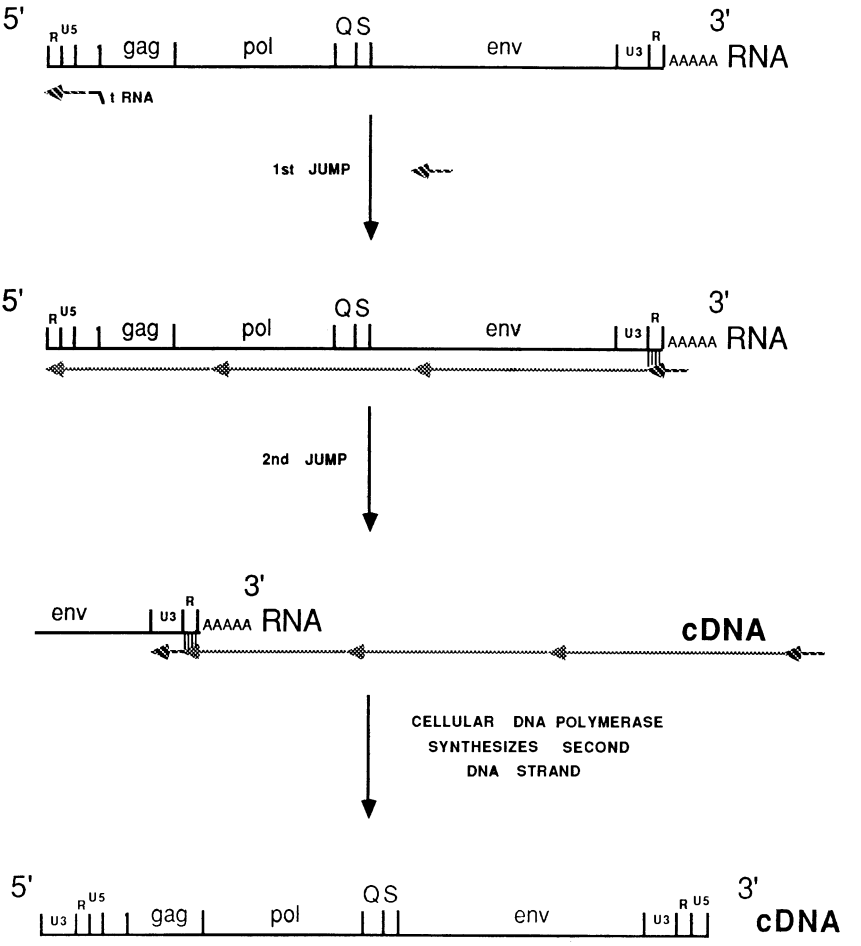


Fig. 2. Replication of the viral RNA by reverse transcriptase

The viral encoded RTase lacks editing functions (exonuclease activities which correct base mismatches) which are characteristic of procaryotic DNA polymerases. This results in a relatively error-prone replication cycle with 1 error in every 10^4 synthesized bases compared to 1 in 10^6 for polymerases which edit their synthesis. Thus, the mutation rate for the retroviruses and lentiviruses is relatively high. Mutations accumulate in the env gene of viruses obtained from persistently infected animals. These variants may have a selective advantage for some form of survival strategy in vivo (39,40,41). Virus cultivated in vitro remains antigenically stable even after numerous subcultivations and no changes occur in the env gene (25). However, when viruses are grown in the presence of immune sera variants resembling those obtained from immune sheep arise rapidly (40,42). These variants have altered neutralization profiles which are reflected by mutations in the env gene (43,44,45). No other phenotype has been identified in cell cultures. However, it is possible that variant viruses with properties other than those affecting neutralization (e.g. those that can replicate more efficiently in macrophages) may be under continuous selective pressure in the infected sheep whose defense mechanisms remain intact throughout the persistent infection.

The complex replication scheme of the viral RNA results in the duplication of the U3-R-U5 sequences at both ends of the viral DNA (Fig. 1). This structure is called the long terminal repeat (LTR) and contains the sequences required for circularization of the viral DNA. Circularization is a prerequisite for integration of the viral DNA into host DNA. In addition, the LTR contains the sequences which control viral RNA transcription. The duplication of these terminal sequences insures against loss of genetic information in the circularization or integration of the viral DNA and also in the transcription of the genomic RNA.

The DNA replication schemes of retroviruses and lentiviruses are very similar to each other but they differ with respect to the ratio of linear to circular DNA that is synthesized during replication. During replication of retroviruses both types of DNA can be detected but the circular form predominates. In contrast only a small portion of the lentivirus DNA becomes circularized and only about 5 copies of these become integrated in each infected cell (34,37,46). However, many more copies (100-200 copies/cell) are found as free double-stranded linear DNA molecules in the nucleus of the infected cell (47). The integrated viral DNA is the most efficient template for viral RNA transcription (48). In the case of the lentiviruses, it is not currently known whether transcription of the linear unintegrated DNA also contributes to the large amount of viral RNA in the infected cell.

Transcription of viral DNA into viral genomic and messenger RNAs

Retroviruses and lentiviruses utilize the eucaryotic cell machinery to transcribe the viral DNA into genomic RNA and mRNAs. This is accomplished by use of cellular RNA polymerase II (Pol II) whose function is to transcribe cellular mRNAs from cellular DNA. The region of the DNA to be transcribed by Pol II contains the nucleotide sequence "TATA" which is called a Goldberg-Hogness Box. It is located upstream (5') of the actual start site of transcription and is the recognition signal (promoter) for Pol II RNA transcription. Some genes have repeated sequences proximal (usually 5') to the TATA box which act (in cis) to increase transcription from the promoter in a tissue or cell specific manner. These nucleotide sequences are called enhancers. In addition, in some cases cellular factors bind to sequences 5' of the TATA box and facilitate binding of Pol II to the DNA. These are called trans-acting factors. The Pol II

binds to the DNA and travels down the DNA in a 5' to 3' direction to the transcription initiation site called the RNA cap site. Transcription proceeds through the gene to a polyadenylation signal (AATAAA) which precedes the termination signal by 15-20 bases. The completed mRNA transcript contains a 7-methylguanosine "cap" at the 5' end and a series of 150-200 adenines at the 3' end (poly (A) tail). In addition, Pol II transcripts are processed at specific sequences called splice sites between which certain RNA sequences are removed. Splice donor (at the 5' end) and acceptor sites (at the 3' end) identify sequences to be removed (introns) from the final mRNA (exons).

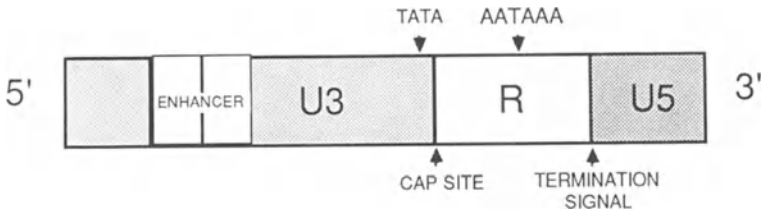


Fig. 3. Lentivirus LTR contains the U3, R and U5 of the viral RNA

Retroviruses and lentiviruses contain Pol II promoters in the viral long terminal repeat (LTR) (Fig. 3). Both enhancer and promoter elements are located in the U3 sequences of the LTR. The cap site for the initiation of RNA synthesis is the 5' boundary of the R region. The polyadenylation site is located in R and the termination of RNA synthesis occurs at the R-U5 junction (Fig. 3).

Transcription of both retroviral and lentiviral DNA produces both full length genomic RNA as well as subgenomic, spliced mRNAs (30,31,32,49,50). Transcription of all retroviral RNAs begins in the 5' viral LTR at the cap site and proceeds to the termination signal at the R-U5 junction in the 3' LTR. The RNAs are "capped" and polyadenylated by cellular proteins and thus resemble cellular messages. However, unlike cellular genes which are either spliced genes or unspliced genes, retrovirus genes are unique in that some of the molecules of RNA remain unspliced whereas others are spliced to produce smaller species. The eucaryotic cell is not known to regulate splicing. Thus, the regulation of splicing must be a function of the virus. Retroviruses transcribe full length genomic RNA. Some of the RNA molecules, however, are spliced to yield a subgenomic mRNA for the env gene.

Lentiviruses follow a similar pattern but the RNA is spliced further to produce multiple subgenomic species. In addition to subgenomic env mRNA small ORFs between the pol and env genes are transcribed into two or three mRNA species (Fig. 4) (30,31,32,51). Five subgenomic viral mRNAs have been identified in visna virus-infected cells in vitro (5.0 kb, a 4.3 kb doublet, 1.8 kb and 1.5 kb mRNAs) in addition to the genomic RNA (9.4 kb) (30) (Fig. 4). At least one of the 4.3 kb mRNAs is the env gene messenger RNA; the function of the other 4.3 kb mRNA is presently unknown. The 5.0 kb mRNA contains the entire Q region and probably produces a protein which regulates viral gene expression. The small mRNAs (1.8 and 1.5 kb) are produced after at least 2 splicing events and contain nucleotide sequences derived from the small ORFs Q and S as well as sequences from the 3' end of the genome (Fig. 4).

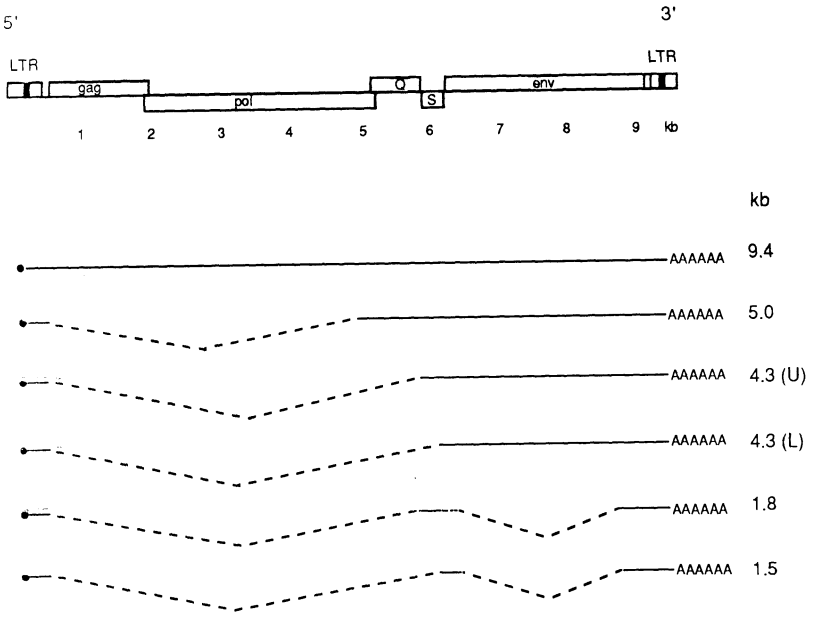


Fig. 4. Transcription of visna virus DNA into mRNAs. The dotted lines indicate introns which are removed by splicing giving rise to the mRNA. The size of each mRNA in kilobases is to the right of the structure.

In addition to the complex pattern of transcription of visna virus there is temporal regulation of transcription. Thus, the smallest mRNA molecule in infected cells is not synthesized until 2-4 h after the other mRNA species had been transcribed. However at 24 h after infection this mRNA is present at the same level as the other viral mRNAs. The function of the protein product of this small mRNA is not known. However it may have a regulatory function not needed early in the replication cycle. One possible function is the

suppression of splicing in order to increase the level of genomic RNA for progeny virus. Another possible function is facilitation of efficient translation of viral proteins by the infected cell.

The visna virus—infected cell contains trans-acting factors which increase expression of any gene which is attached to the visna virus enhancer/promoter element and sequences that include the virus cap site (52). These trans-acting factors enhance the transcription of LTRs of both the visna virus and CAE viruses. In addition, post-transcriptional activation of the visna LTR but not the CAEV LTR is observed. CAEV does not induce trans-acting factors (53). However, its LTR is responsive to those produced by visna virus. The trans-activation of virus gene expression may be responsible for rapid mobilization of the cell transcriptional machinery for viral transcription which results in rapid accumulation of viral RNA in the infected cell. The post-transcriptional effect may result in the more efficient translation of viral protein products. These effects suggest that trans-acting factors are important in the replication of visna virus in vitro. However, these factors are not required for lentivirus replication since CAEV replicates as well as visna virus in vitro and both viruses transcribe equivalent levels of viral RNA at times of peak CPE. Despite this, visna virus is highly cytolytic in vitro while CAEV is not. Thus, it is tempting to speculate that these trans-acting factors may be involved in this highly cytolytic type of replication. Further, the importance of these trans-acting factors in vivo is questionable since all ruminant lentiviruses, including CAEV, cause persistent infection and disease in their natural hosts, irrespective of their ability to produce trans-acting factors in cell culture.

The small mRNAs of visna virus are analogous to those of HIV. These mRNAs in HIV produce proteins which regulate the

gene expression of the virus in trans at a post-transcriptional level (TAT-III gene) (51,54,55,56,57). They are also thought to have regulatory functions in splicing of viral RNAs (TRS gene) (58) and in the translation of viral structural genes (ART gene) (59). Thus, these small mRNAs appear to be important for the complex gene expression of lentiviruses and may play a role in the restricted gene expression observed for the lentiviruses in vivo.

Translation of viral mRNAs and processing of viral polypeptides

The genomic RNA is the mRNA for the gag gene and the pol gene. Translation of this RNA produces a gag precursor protein of 55,000 daltons (28,60) which is cleaved into the three core proteins p16, p25 and p14 (encoded 5'-3' on the gene). A gag-pol precursor protein is also translated from the genomic RNA and is of 175,000 daltons. This precursor is processed by a viral protease (5' end of pol ORF) yielding the RTase as well as another protein, the endonuclease/integrase which is involved in the integration of the viral DNA into the host cell DNA. The gag and the pol genes are the most highly conserved genes in both retroviruses and lentiviruses. By both nucleotide sequence homology and antigenic cross reaction these proteins are highly conserved among the ovine and caprine lentiviruses. (18,35,61,62,63,64).

The env precursor protein of 115 Kd is translated from one of the two 4.3 kb mRNAs. This protein is glycosylated (150,000 daltons) and is cleaved into a mature glycoprotein of 135,000 daltons. The sequence of the env gene of visna virus is similar to that of HIV in that it contains a cleavage site within the glycoprotein which would produce outer membrane and transmembrane proteins. However, the major glycosylated component of visna virus is the uncleaved 135,000 dalton glycoprotein with minimal detectable levels of

the cleaved inner and outer membrane proteins. The lentivirus envelope glycoprotein contains the epitopes for binding to cellular receptors and for inducing neutralizing antibody. The env genes of different lentiviruses share nucleotide sequence homology in specific regions, suggesting that there are conserved and variable domains in the protein (63).

Currently, protein products of the 5.0 kb RNA, the second 4.3 kb mRNA and 1.8 and 1.5 kb mRNAs have not been identified as yet. These mRNAs have been sequenced and sequence-specific peptides synthesized and used to immunize rabbits. These anti-sera are currently being used as probes to identify homologous proteins in infected cultures. The amino acid sequence of these proteins are highly basic suggesting that they might be nucleic acid binding proteins.

Homology between visna virus and CAEV and the effect of serology

Despite wide differences in biological properties between visna and CAE viruses these viruses are related antigenically, with sharing of epitopes in the core as well as the envelope proteins (18,20,35,61,65). The LTRs of the two viruses have little overall homology but highly conserved sequences are present in the enhancer and promoter regions (53). In addition, there is extensive nucleotide sequence homology in the gag-pol genes and more limited homology in the env gene (35,63). These homologies are reflected in the serologic responses of infected animals whose antibodies recognize core proteins of both visna and CAEV in immunodiffusion and ELISA tests. Binding antibodies to glycoprotein antigens are also shared. However, virus neutralizing antibodies induced by epitopes in the glycoprotein are more restricted and tend to be virus strain specific. The virions appear to have multiple neutralization epitopes, only some of which are exposed to the immune

system. The arrangement of these epitopes is specified by nucleotide sequences in the env gene and rearrangement results when certain mutations occur in this gene.

Permissive tissue culture systems have been used to quantitate and identify viral nucleic acids appearing during replication of CAE and visna viruses. Virus specific RNA was harvested at sequential intervals from GSM cultures, inoculated with CAEV at a MOI of 1, and quantitated by RNA dot blot analysis. This showed that viral RNA began to accumulate in the cells by 24 h after inoculation. The RNA levels reached peak values of approximately 16,000 genomic copies per cell (Pyper, unpublished observations) by day 4, and remained at this level until day 7. The pattern of viral-specific DNA accumulation in CAEV-infected GSMs paralleled RNA accumulation (Pyper, unpublished observations).

The levels of viral DNA and RNA of visna virus in permissively infected cell cultures and in cells from infected animals have been quantitated more thoroughly than those of CAEV. Viral RNA in cultures was measured by dot blot analysis and in tissue by in situ hybridization using ^{35}S labeled cloned DNA probes. In the permissive SCP cell cultures there were 300-500 copies of viral DNA per cell at peak virus production (47). The viral RNA at this time was approximately 20,000 copies per cell (66). Much lower levels of virus gene expression were seen in the infected animal, especially of RNA (and protein). In paraffin sections of choroid plexus from an infected lamb, 18% of nuclei contained viral DNA, but only 0.1% of cells expressed p30, the major core protein of the virus (67). In another experiment only 1-3% of cells contained proviral DNA or viral RNA; the copy numbers in positive cells were approximately 65 copies of proviral DNA and 140 copies of viral RNA (68).

Virus Replication in Natural Target Cells

Recent work has shown that monocytes and macrophages are the primary targets of the lentiviruses. Early work with visna virus had shown that white blood cells are infectious (69,70). Subsequent studies showed that monocytes are the only cells of the peripheral blood leukocytes which are infected (71). In tissue culture, both monocytes and macrophages can be infected with virus; the permissiveness of infection increases as the monocytes differentiate into macrophages (17,71,72,73). Although monocytes can be infected, they remain latently infected and only produce virus upon differentiation into macrophages (71).

In situ techniques have been used in attempts to quantitate the numbers of copies of viral genome per cell and the number of infected cells in various target organs. These experiments have shown that replication in monocytes is severely restricted (73,74). Two weeks after experimental inoculation of sheep, only 0.02-0.6% of monocytes were infected and the copy numbers were low (a few DNA copies per cell, and up to 50 RNA copies per cell) (74). In vitro experiments correlated virus replication with the stage of maturation of the monocyte-macrophage (73). Few monocytes could be infected and those infected had very few copies of viral nucleic acid. However, as the monocyte matured into macrophages, they became more susceptible to infection and more permissive for replication. However, production of infectious virus did not occur until the cells were fully mature.

Pulmonary alveolar macrophages from experimentally infected lambs have also been assayed for virus replication. For these experiments Gendelman et al. (75) used a double-labeling technique employing in situ hybridization to identify viral RNA and immunocytochemistry to identify macrophages. The double-labeling technique showed that all lavaged cells containing viral RNA were alveolar macrophages

(copy numbers ranging from 50 to 1000 copies per cell two to three weeks after inoculation). These cells were hosts for viral replication since both viral RNA and DNA could be detected, although DNA levels were not quantitated. However, only 1% of cells containing viral RNA produced virus (determined by infectious center assay) (75,76).

Analysis of tissue sections from infected sheep, using these two labelling methods (75), showed that: 1. More than 90% of cells in various tissues containing viral RNA also had the macrophage marker; 2. The highest concentration of viral RNA was found in organs that normally develop inflammatory lesions and lymph nodes draining these organs; 3. No viral RNA was found in Kupffer cells, implying that only subsets of macrophages are susceptible to infection; 4. Some of the monocyte-macrophage precursors in the bone marrow had viral RNA in low copy numbers. Fewer copies of viral RNA were present in the bone marrow precursors than in macrophages in the spleen and lung. This result is consistent with the *in vitro* experiments which showed that viral replication depends on the maturational state of the macrophage.

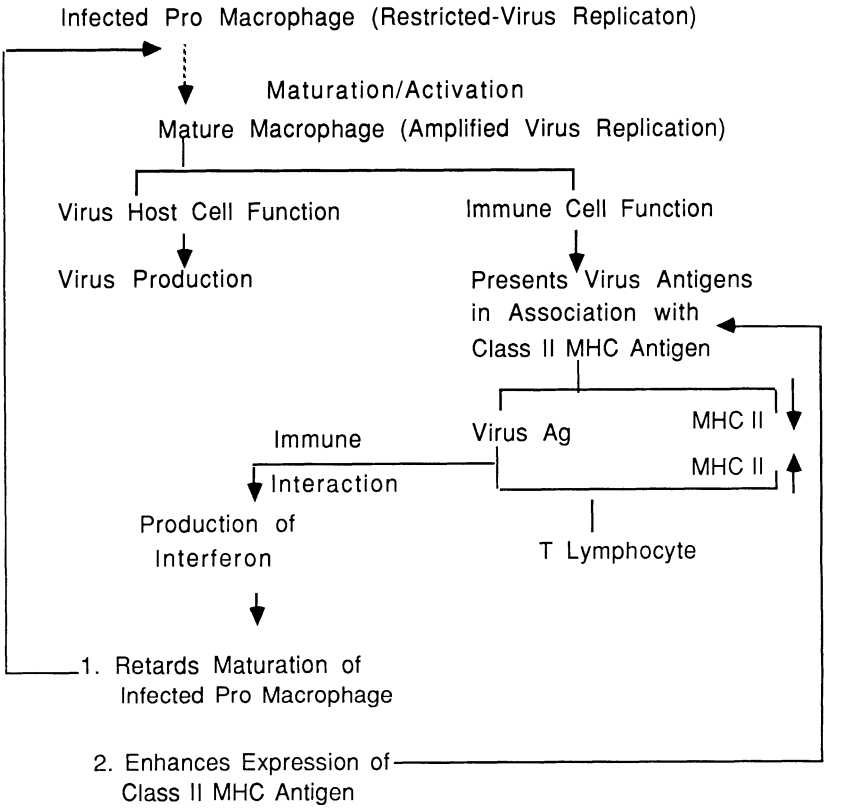
Macrophages were the only infected cells in target tissues. Since lymphocytes were not infected, the proliferation of these cells in areas contiguous to infected macrophages may be due to an immunological reaction resulting from presentation of viral antigens within the context of class II major histocompatibility antigens (Ia) by the infected macrophages. A double-labeling technique using anti-Ia antibodies and a probe for viral nucleic acid showed that in lung sections approximately 30% of the cells containing viral RNA also expressed Ia antigens (6). This suggested that in the lung infected macrophages were immunologically activated and were presenting antigens. A similar pattern was seen in the mediastinal lymph node draining the lung and also in the spleen.

Immunologic Functions of the Infected Macrophage

The observation that the maturational state of the monocyte-macrophage was important in determining levels of viral gene expression and virus production led to the hypothesis that the maturing macrophage might produce an interferon which would inhibit virus replication in other populations of macrophages. Supernatant fluids from infected cultures of macrophages, SCPs, lymphocytes and GSMs did not have interferon activity (measured by protection of GSM cultures against CPE caused by vesicular stomatitis virus). However, when lymphocytes were added to the infected macrophage cultures, interferon activity was detected 12-24 hours later in the supernatant fluid. This interferon was specific for sheep or goat macrophage cultures infected with either visna virus or CAEV. Preliminary studies suggest that T lymphocytes and not B lymphocytes are responsible for the interactive induction (7).

The supernatant fluid containing this interferon has two biological properties which are probably important in the disease process. First, the fluid inhibits the maturation of monocytes to macrophages in culture (77). This inhibition effectively limits the production of infectious virus since virions are only produced by mature macrophages. This mechanism may be important in controlling virus replication in the animal. Secondly, this interferon induces expression of Ia antigens in the macrophages (6). Lentiviruses do not cause expression of Ia in the macrophages that they infect. Induced expression of Ia by IFN in macrophages producing virus antigens may therefore be a step-wise process:

MECHANISM OF RESTRICTED REPLICATION OF THE RUMINANT LENTIVIRUSES



The viral antigen responsible for triggering the production of interferon has not been identified as yet. The antigen is not present in virus particles because addition of purified inactivated virus to Ia positive macrophages does not result in interferon production. Rather, presentation of

the antigen requires infection by the virus (7). The antigen may therefore be a precursor protein or a non-structural protein encoded by one of the viral mRNAs whose function is yet to be determined. This antigen may be the key to the fundamental pathogenic difference between the lentiviruses which cause inflammation and the retroviruses which may cause oncogenesis.

A possible scenario for pathogenesis is that lymphocytes in the presence of the infected macrophages produce the lentivirus-specific interferon. In turn, this interferon induces Ia expression on both infected and uninfected macrophages. This interferon may be very localized since interferon activity has not been detected in infected animals (7). The dual activities of the interferon (1) restriction of virus replication by inhibition of maturation of monocytes into macrophages, and (2) stimulating expression of Ia antigens in infected macrophages, could explain the slow replication of the virus in vivo and the constant, immunologically specific antigen stimulation that leads to lymphoproliferative lesions in target tissues.

Determinants of Infection in Select Macrophage Populations

In view of the basic similarities in mechanism of replication of CAEV and maedi/visna virus and the common immunopathologic pathways of inflammation it seemed surprising that there are sharp differences between the diseases observed in goats (slowly progressive arthritis in adults and rapidly progressive encephalitis in kids) and those in sheep (dyspnea resulting from interstitial pneumonia). These differences in clinical expression of infection may be due in part to differences in the ability of different macrophage populations to support virus replication. Thus, synovial macrophages of goats may be as permissive for virus replication as alveolar macrophages in sheep. This would explain the high incidence of arthritis

and pneumonia respectively, in the two species. More recent findings in naturally infected animals however have shown that disease in sheep and goats may not be as clearly definable by tissue specificity as originally thought. Rather, disease in either species may involve any of the four organ systems: the CNS, lungs, joints and mammary glands. Thus, mastitis and interstitial pneumonia are common to both sheep and goats (1; ON, unpublished observations). Further, synovitis has been identified as the major disease entity in some isolated sheep flocks (ON, unpublished observations).

In addition to host species differences which may determine tropism of the virus for different macrophage populations, different breeds of sheep appear to have genetic determinants that regulate the permissiveness of specific macrophage populations for support of virus replication. A clear-cut example of this is the difference between British and Icelandic sheep with respect to the ability of lung and brain cells to support virus replication. Whereas lung cells from sheep of both breeds support virus replication (and thus develop pneumonia) brain cells of only Icelandic sheep are capable of replicating virus (P.G.E. Kennedy, O. Narayan et al., unpublished observations). This correlates with the natural disease picture since Icelandic sheep were more susceptible to neurologic disease (visna) than non-Icelandic sheep (1,78,79).

Factors associated with natural spread of these viruses may have a role in determining the selectivity of macrophage populations that are infected. The practice on dairy farms of feeding goat kids milk pooled from all lactating does (some of which may be latently infected) greatly increases the chances of infecting the young animals <u>en masse</u>. The unusually high incidence of encephalitis in kids reflects this possiblity. Similarily, the crowded housing of sheep in poorly ventilated barns during winter months may potentiate infection of alveolar macrophages leading to the natural high

incidence of pneumonia in these animals. Thus, the tropism of lentiviruses for particular macrophage populations (and disease) may depend on genetic factors in the virus and the host and, in addition, environmental factors which may enhance infection of particular subpopulations of cells in specific groups of animals.

While the early inflammatory lesions in the synovia, mammary glands, lungs and the brain can be explained on the basis of virus replication in organ-specific macrophages, followed by the cascade of immunopathological events described above, the mechanisms for the late dystrophic lesions in cartilage and bones in joints of arthritic goats and the late demyelinating lesions in brains of sheep with visna are not understood. However, it is possible that continuous insult to tissues by proteases and cytokines produced constantly by immunologically specific inflammatory cells could lead to gradual progressive deterioration of normal tissues into which these cells migrate.

Acknowledgements

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9

SHEEP PULMONARY ADENOMATOSIS: CLINICAL, PATHOLOGICAL AND EPIDEMIOLOGICAL ASPECTS.

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ABSTRACT

Sheep pulmonary adenomatosis (SPA) is a contagious lung tumour of sheep and, rarely, goats which manifests itself clinically as a progressive pneumonia. Two secretory epithelial cells are transformed, the type II pneumocyte in the alveolus and the cell of Clara in the terminal bronchiole. SPA is the most common tumour of sheep and occurs in many countries. Within affected flocks, it is estimated that SPA occurs in up to 30% of the sheep and is responsible for 50% of the mortality.

INTRODUCTION

Sheep pulmonary adenomatosis (synonyms, jaagsiekte, ovine pulmonary carcinoma) is a contagious disease of sheep and goats resulting from the development of an adenocarcinoma in the lungs. The disease has been recognised since the early 19th century (1) but it was not until almost 100 years later that Aynaud (2) postulated that SPA might be a tumour.

This article will review the clinical, pathological and epidemiological features of the disease that have contributed to the view that SPA is a contagious tumour.

Clinical Signs

Epidemiological observations have indicated that SPA normally has a long incubation period (3). Consequently the disease is rarely seen until sheep are more than 2 years old with the peak incidence occurring at 3-4 years of age (4). In contrast to this typical picture are those rare cases in lambs 3-6 months old in which the incubation period must be much shorter (personal observations).

These epidemiological observations of naturally occurring SPA have been borne out by experimental transmission studies. Experimentally, clinical illness develops only after several months following

inoculation of tumour homogenate or lung fluid by a variety of routes into adult sheep or lambs several months old (5). However, in very young lambs, clinical illness can develop more rapidly, usually in 3-6 weeks (6). Clearly the age at time of infection strongly influences the incubation period.

Sheep affected with jaagsiekte are likely to show clinical signs only when the tumour volume becomes sufficiently large to interfere with normal lung physiology and function, although small areas of tumour tissue may be present without producing obvious clinical signs. Signs are those of progressive respiratory embarrassment. Such clinical signs are most obvious following exercise, hence the name "jaagsiekte" which is derived from Afrikaans meaning "driving sickness" (jagt = driving, ziekte = sickness). The degree of tachypnoea, hyperpnoea, and even dyspnoea in terminal cases or after excessive exercise, reflects the extent of the tumour and loss of normal functional alveolar architecture. A striking, and unique, feature of SPA is the accumulation of fluid within the respiratory tract. The presence of this fluid may be detected on auscultation as moist rales and sibilant rhonchi. In advanced cases the respiratory sounds may be audible without the aid of a stethoscope. When the hindquarters of the affected sheep are raised and the head lowered a frothy seromucoid fluid runs from the nostrils (Fig. 1). As much as 300 ml may be collected, although quantities less than 50 ml are more usual and in long-standing cases it may be impossible to obtain fluid by this technique. A surprising observation is that, despite the presence of this fluid throughout the respiratory passages, coughing is never a prominent sign.

Affected sheep remain alert, afebrile and have a good appetite, though progressive loss of weight is obvious. Death inevitably occurs within a few weeks once signs become obvious. A few animals may survive for many months if they are housed and receive close attention.

Pathology

I. Natural SPA

Necropsy findings

Early reports provide the most graphic descriptions of the appearance of the lungs in clinically-affected sheep (7, 8). The lungs usually are considerably enlarged and, because of the amount of

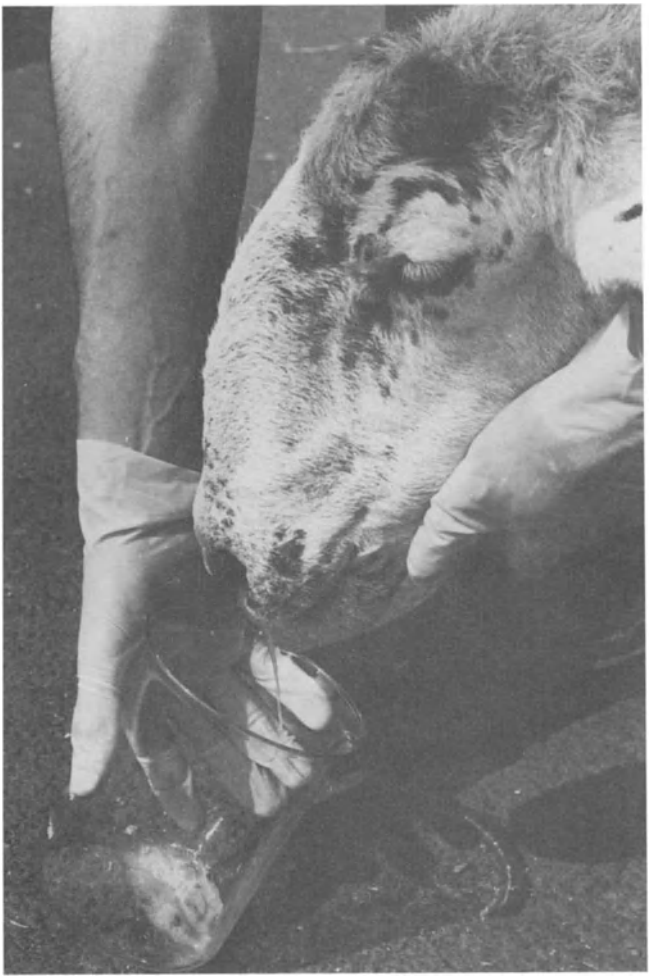

Fig. 1
Fluid nasal discharge from a sheep with SPA
Reproduced from "Diseases of Sheep" Ed. W.B. Martin, by courtesy of Blackwell Scientific Publications

consolidation and associated lack of aeration, they may be up to five times as heavy as unaffected lungs. In advanced cases, the most dependant, or ventral, parts of the lungs are completely consolidated, and this often includes the right apical and both cardiac lobes, the intermediate lobe, and the lateral margins of the posterior, or diaphragmatic, lobes (Fig. 2). In early cases, solitary tumour nodules may be the only evidence of disease; these are often found in the dorsal aspect of one or other of the diaphragmatic lobes. Moderately advanced

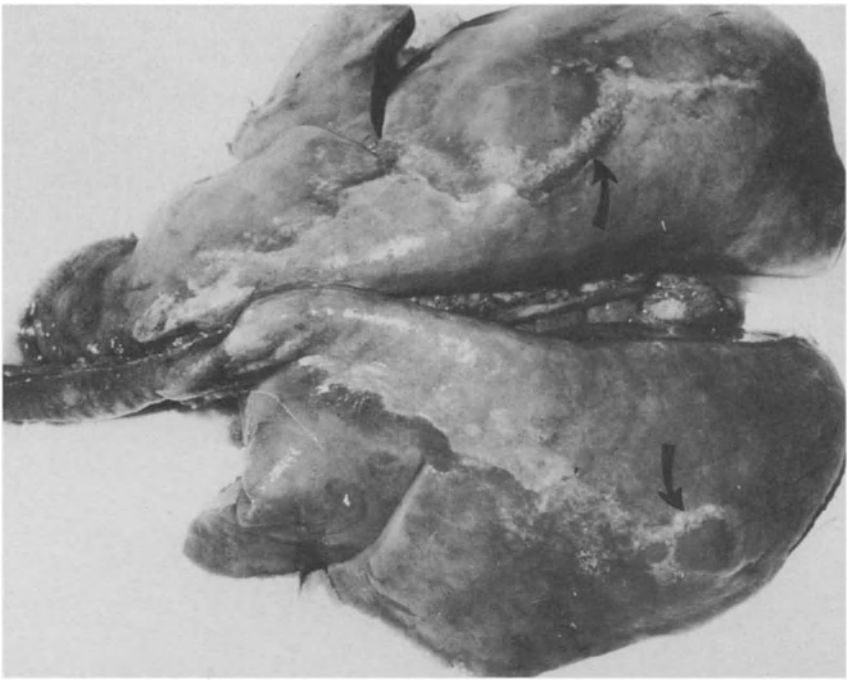

Fig. 2. Sheep lungs with typical SPA lesions. Arrows indicate emphysematous zones between affected and normal lung. From W.B. Martin et al (1979) (11). Reproduced from Comparative Immunology, Microbiology and Infectious Diseases, by courtesy of Pergamon Editors.

cases show limited lesions; the right anterior lobes are most frequently involved, and their appearance in abattoir specimens is often sufficiently characteristic to establish a diagnosis. These localised lesions expand as the disease progresses, and usually have become confluent by the time clinical symptoms are recognisable.

Neoplastic areas are solid, slightly raised above unaffected, adjacent lung, and are grey or light purple with a translucent appearance. Quite often a narrow zone of emphysematous lung separates the tumorous from the normal parenchyma (Fig. 2). The tissue feels moist and is very friable. When neoplastic lung is incised, frothy fluid may pour from the bronchioles but the tumour tissue seldom exudes fluid. The cut surface usually shows numerous small, slightly-elevated nodules in section. Advanced cases are seldom uncomplicated,

and a common finding is chronic pleurisy, with fibrous adhesions which may make it difficult to remove the lungs from the thorax. Pulmonary abscessation and bacterial pneumonias, particularly pneumonic pasteurellosis, are the most frequent complications. The bronchial and mediastinal lymph nodes are usually enlarged and have hyperplastic cortical zones. Occasionally, small metastatic lesions can be seen in the cut surfaces of regional nodes. There are few reports of any extrathoracic manifestations of the disease (9) and the available evidence suggests that instances of extrathoracic spread are very rare.

Microscopic pathology: light microscopy

Characteristically, the pulmonary air spaces or alveoli are continuously lined by cuboidal or columnar cells (Fig. 3), often forming papilliform ingrowths which produce an elaborate adenomatous pattern. Cells in mitosis can be seen in most lesions but are not numerous, perhaps reflecting the slow rate of development of the tumour. The neoplastic cells are ranged on a fibrous framework which gradually becomes thicker and more organised until, eventually, the acinar spaces are widely separated and little resemblance to normal lung remains. At the interface between tumour and normal lung, however, it is usual to find more nodular foci supported by delicate stromal frameworks; the constituent cells of these foci have unstained or vacuolated cytoplasm, compared with those of more advanced lesions, where the cells have dense, eosinophile cytoplasm (10). These vacuolated cells are rich in glycogen, and may be in an intensely active state of development; thus, lesions composed of these cells may represent areas of rapid spread. Another feature of SPA commonly seen concurrently with the nodular alveolar lesions occurs in the terminal bronchioles, where multiple polypoid ingrowths, often very extensive, arise from the bronchiolar epithelium (Fig. 4) and cause partial obstruction with bronchiectasis (11). Additionally, nodular or even diffuse masses of myxomatous tissue (Fig. 5), presumably of mesodermal derivation, may be found in SPA tumour lesions (7, 10, 12). Similar lesions have been described in regional lymph nodes and in extrathoracic tissues (9), though metastatic lesions of an adenomatous character (Fig. 6) can also occur (9, 12). A characteristic feature of non-tumourous lung in SPA cases is the presence of copious exudates of large free macrophages in alveoli (Fig. 4). These cells have vesicular nuclei and extensive, vacuolated

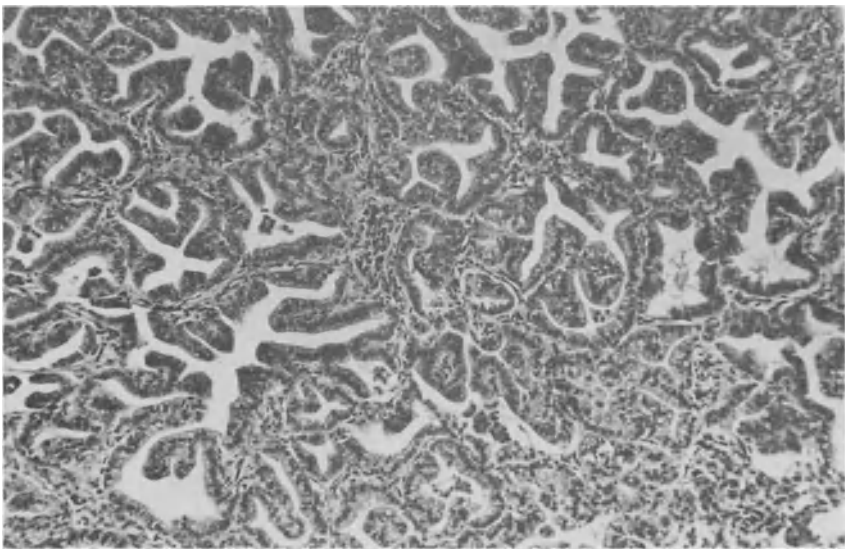

Fig. 3. Typical adenomatous structure of SPA tumour

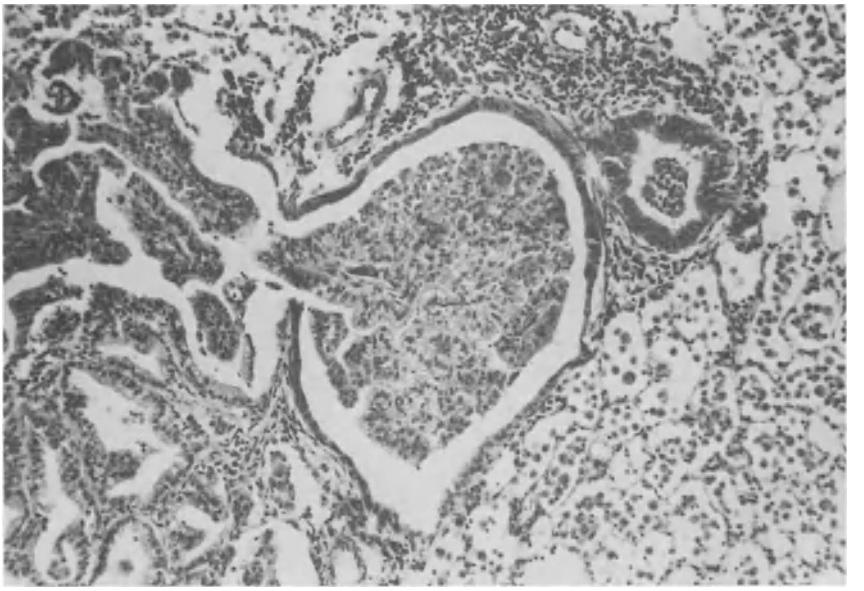

Fig. 4. Intrabronchiolar tumour polyp in SPA (centre). On the right are unaffected alveoli containing pulmonary macrophages. From W.B. Martin et al (1979) (11). Reproduced from Comparative Immunology, Microbiology and Infectious Diseases, by courtesy of Pergamon Editors.

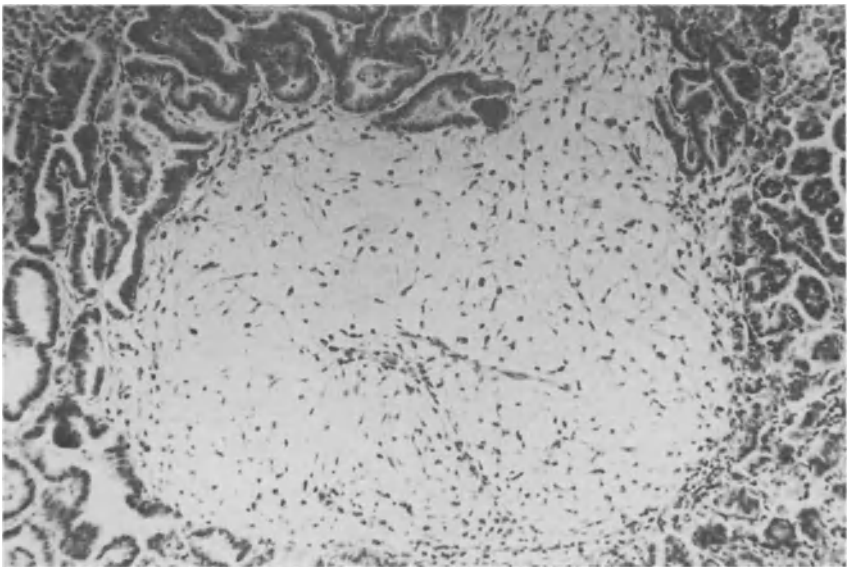

Fig. 5. Structure of myxomatous nodular growth in SPA tumour

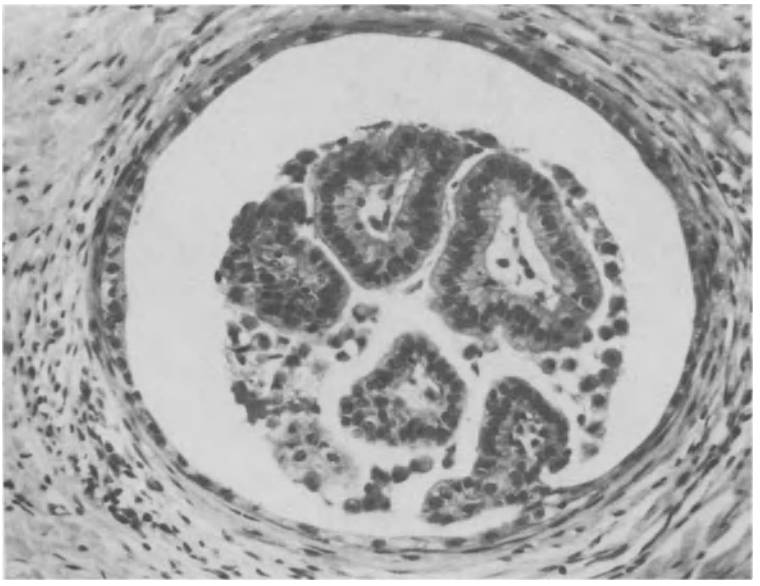

Fig. 6. Adenomatous SPA tumour metastasis in regional lymph node. (From the collection of the late Dr. D.I. Nisbet).

or "foamy" cytoplasm, rich in the enzyme B-galactosidase. Infiltrates of lymphoid and plasma cells may be seen to a greater or lesser extent in the interstitium or around bronchioles and pulmonary vasculature throughout the lungs in SPA cases, but it is not known whether the presence of these cells is due to intercurrent infections or to the disease process itself. The regional lymph nodes have hyperplastic, reactive cortices but no other specific changes. Where complication with other pulmonary infections has occurred, the tumour lesions may be obscured by other pathological changes. The possibility of concurrent infection with SPA and maedi-visna must always be considered.

Ultrastructure of naturally-occurring SPA

The findings in published ultrastructural studies of naturally-occurring SPA are not always entirely consistent, but this is probably partly due to the inevitable variation in the age of the lesions investigated in any series, and perhaps to the fact that both rapidly-developing and long-standing lesions can co-exist in the same sheep. However, there is general agreement on the appearance of the constituent cells of the alveolar form of the tumour (10, 12, 13, 14). These cells have well-developed desmosomal junctions, little or no interdigitation of their lateral membranes, and numerous prominent microvilli on their free surfaces. Multiple apical vacuoles or cytosomes containing lamellated myelinoid bodies have been described in these cells (10, 12, 13), causing them to be regarded as modified or transformed type II or granular pneumocytes. Similar cells in early SPA lesions also contained clusters of microtubules, which were not observed in more advanced lesions (14). Large glycogen deposits have been observed in tumour cells (12, 14) but some authors (13) found only small amounts. Intrabronchiolar proliferations are composed mainly of non-ciliated cells with sparse or vestigial microvilli, abundant rough-surfaced endoplasmic reticulum and apical membrane-bound electron-dense granules. These cells have been designated as being analogous to the cells of Clara, which contribute components to pulmonary surfactant (13). Intracellular A-type virus particles have been detected from time to time in cells in the alveolar, but not the intrabronchiolar, lesion (14) while occasional C-type particles have been detected budding from the outer membranes of cells in the stroma (14).

II. Experimental SPA

For many years the main constraint which hampered experimental investigations of the pathogenesis of SPA was the inordinate length of incubation periods when homogenates of cells from naturally-occurring tumour tissue were used to inoculate sheep. However, eventually the disease was transmitted by inoculating sucrose density gradient fractions with reverse transcriptase (RT) activity, obtained from tumour supernates (15). Similar RT activity was later demonstrated in lung fluid from sheep with clinical SPA, and when this was concentrated and used to inoculate newborn lambs (6), the dramatic reduction in the timecourse of the disease from many months to a few weeks made pathogenesis studies possible. By 10 days after inoculation, small intrabronchiolar and intra-alveolar polypoid ingrowths had developed (6), and lambs killed two weeks later still were dyspnoeic and had disseminated tumour lesions throughout their lungs (Fig. 7). Light microscopy of the lesions showed that involvement of the terminal bronchioles was widespread (Fig. 8), and nodular lesions, composed of acinar structures either derived from alveolar epithelium, or which had spread by extension from the bronchiolar tumours, were numerous (6). Precisely the same sequence of events occurred when suspensions of type II pneumocytes which had been cultured from naturally-occurring SPA tumours were used to inoculate newborn lambs (Fig. 9). The cells comprising the experimentally-induced tumours were either cuboidal, with central spherical nuclei and dense, eosinophile cytoplasm, or narrow columnar cells with apical nuclei and clear unstained basal cytoplasm (Fig. 10). Both of these cell types contained large amounts of PAS-positive amylase-degradable granular material in their cytoplasm, indicative of a high glycogen content (16). The fibres of the stromal framework of many lesions were embedded in an acid mucopolysaccharide ground substance (16).

Ultrastructure of experimentally-induced lesions

Scanning electron microscopy of early tumour lesions has shown some detail of the topography of both alveolar and intrabronchiolar lesions (16). The former can be seen as polypoid projections into alveolar spaces (Figs. 11, 12), composed of cells which possess numerous, prominent microvilli (16, 17). In contrast, intrabronchiolar lesions are covered mainly with cells morphologically indistinguishable from the

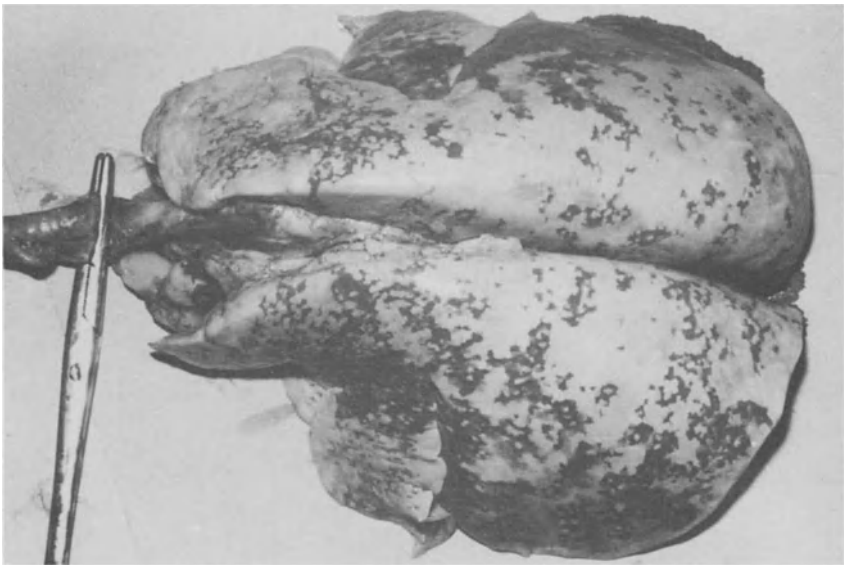

Fig. 7. Distribution of SPA tumour lesions in a 23 day-old lamb inoculated 6 hrs after birth with concentrated lung fluid from a natural case of SPA.

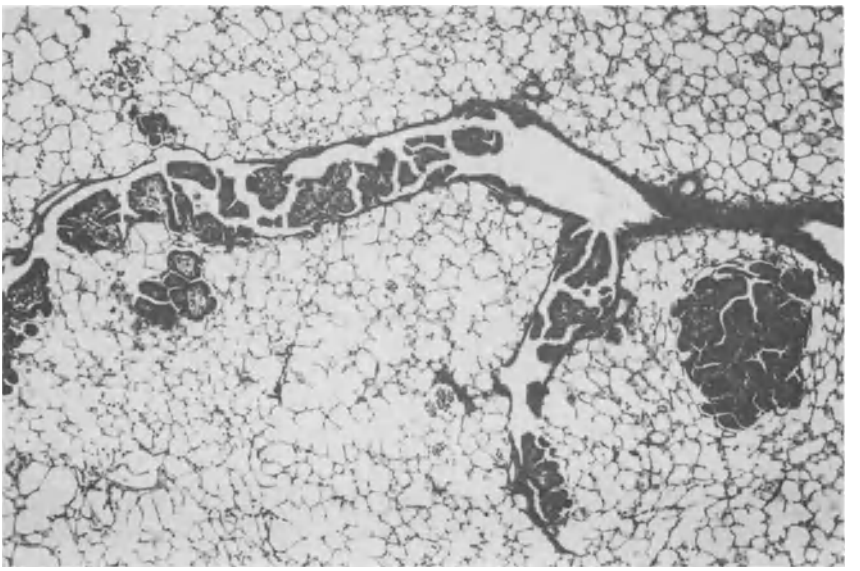

Fig. 8. Microscopic pathology of lung shown in Fig. 7, showing extensive tumour spread along the bronchiolar tree. From J.M. Sharp et al. (1983) (6). Reproduced by courtesy of the Editors, Archives of Virology (Springer-Verlag, Wien/New York).

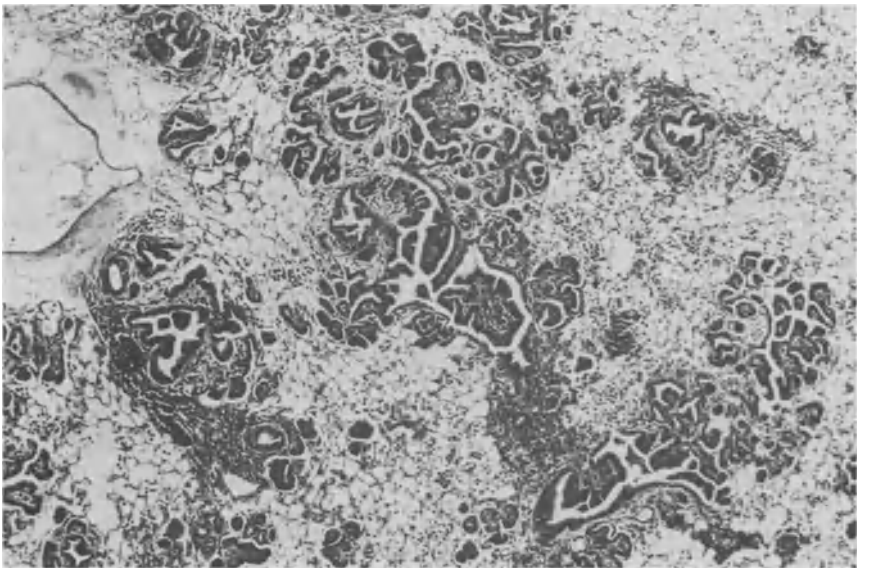

Fig. 9 Extensive tumour spread in a 120 day-old lamb, following experimental inoculation at birth of 10^8 JS7 cells, a cell line derived from SPA tumour tissue after 16 passages in tissue culture. Reproduced by courtesy of Dr. F.A. Jassim, Moredun Research Institute.

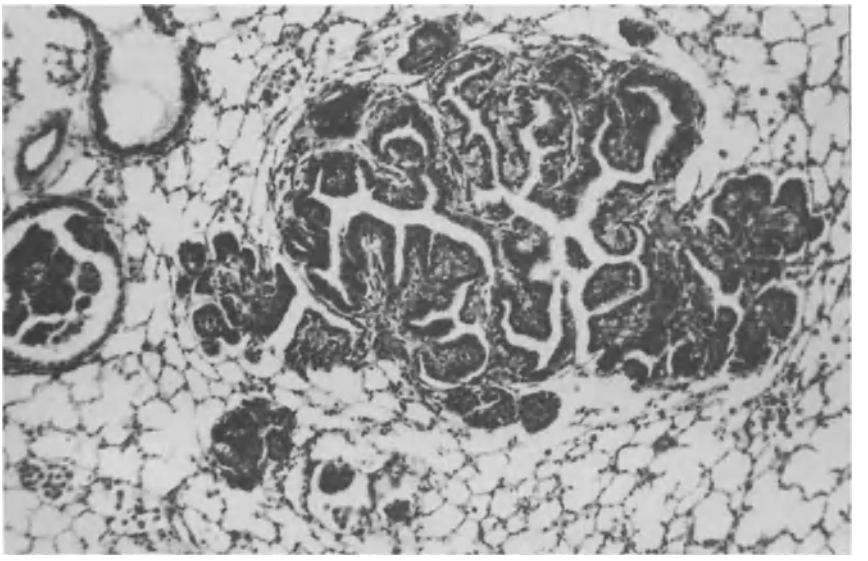

Fig. 10. Experimentally-induced SPA tumour, 23 day-old lamb. Both intensely-stained or virtually unstained cells are present in the same lesion.

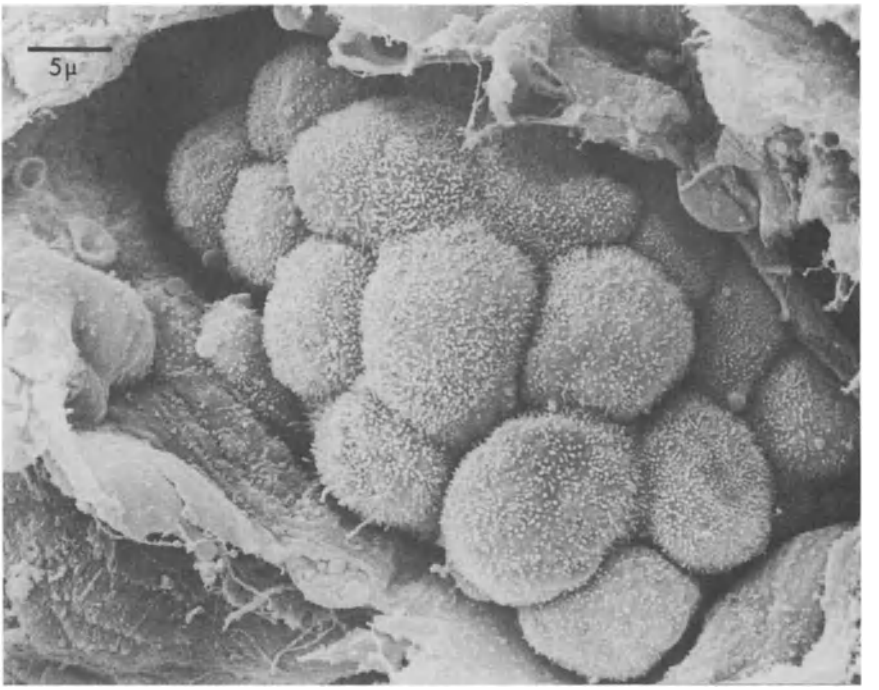

Fig. 11. Scanning electron micrograph of intra-alveolar tumour polyp, 23 day-old lamb

non-ciliated bronchiolar lining cells of normal lung. The luminal surfaces of these tumour cells possess sparse and vestigial microvilli (Fig. 13). Transmission electron microscopy (Figs. 14, 15) confirms the close resemblance between the principal cell type in the alveolar tumours and the type II pneumocyte (16, 17). This judgement is based on the presence of prominent microvilli, tight junctions between cells and the apical cytosomes containing either electron dense, electron lucent or myelinoid lamellated granules (17). The nuclei of these cells tend to be central and spherical, with peripheral chromatin, though basal nuclei are found in some cells. Tumour cells contain hypertrophic mitochondria and moderate amounts of endoplasmic reticulin (17). Large amounts of glycogen were found in 23 day-old tumour cells (15), though the presence of glycogen does not seem to be a constant feature (17). Furthermore,

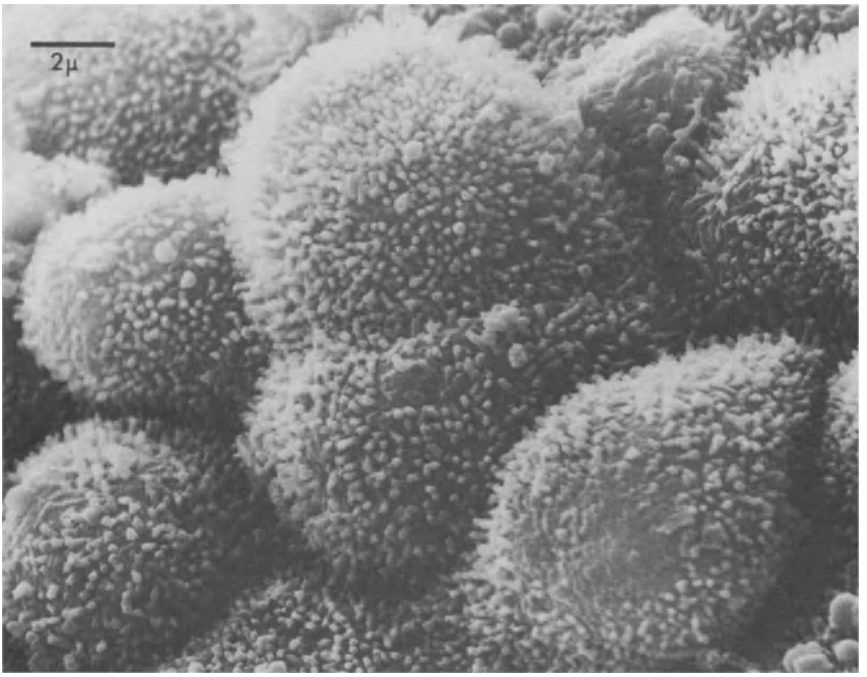

Fig. 12. Higher power of intra-alveolar polyp, showing the prominent microvilli on the apical surfaces of the tumour cells.

in the early stages of tumour spread in young lambs, numerous intracellular A-type and extracellular B or D-type particles have been demonstrated in tumour lesions composed of these cells (6). Other cells which have been described in early tumours include a cuboidal cell with prominent microvilli, numerous mitochondria, well-developed endoplasmic reticulum but no apical cytosomes and very little glycogen (16). These cells, which were thought to be immature type II cells, also had A-type particles in their cytoplasm (16). Less frequently seen were columnar cells with apical nuclei and no cytosomes, though some had apical electron-dense granules. Basally, these cells contained dilated cisternae enclosing structureless material of low electron density. Because they had vestigial microvilli, apical granules and were seen close to the bronchioles, it was suggested that they might be immature

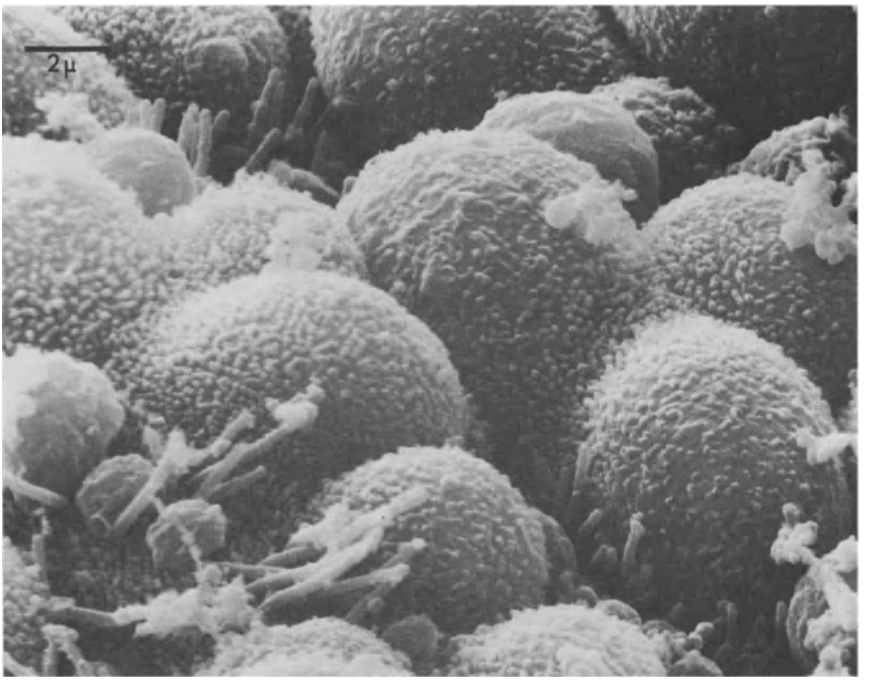

Fig. 13. Non-ciliated tumour cells of intra-bronchiolar polyp, 23 day-old lamb. These have vestigial surface microvilli, compared with those on alveolar tumour cells (Fig. 11). Occasional ciliated bronchiolar cells are also present.

Clara cells (16). No virus particles were ever seen in these cells. These experimental studies have gone some way towards corroborating the findings of earlier ultrastructural studies on naturally-occurring SPA, in that they have identified several of the main target cell-types for virus invasion and replication.

Epidemiology

Distribution and prevalence

Since the earliest descriptions of jaagsiekte in South Africa (1) and SPA in Britain (18), the disease has been recorded in many countries throughout the world (3, 5). Recent additions to the list have been Northern Ireland (19), Canada (20), the USA (12), Peoples Republic of China (21) and Mexico (22). Australia and New Zealand continue to remain free.

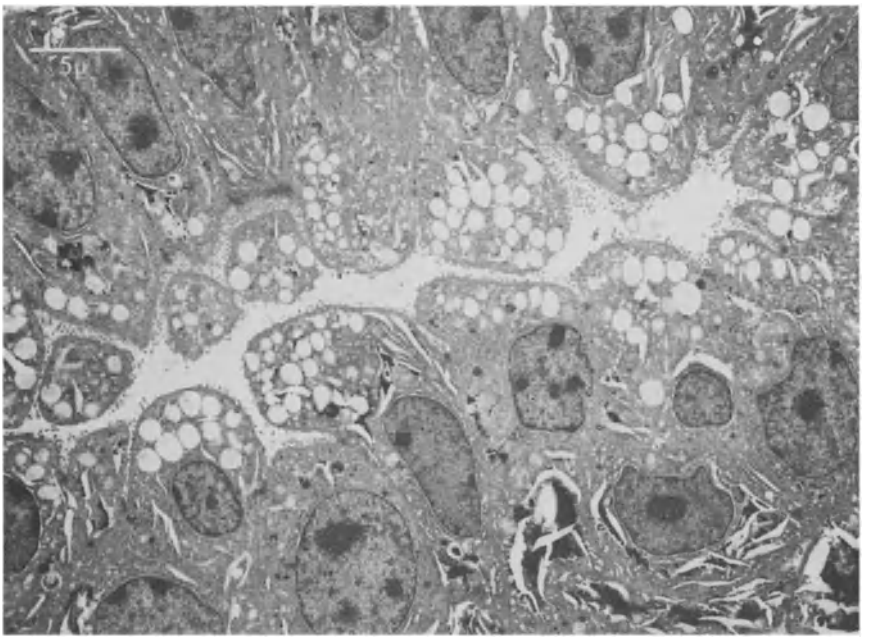

Fig. 14. Low-power electron micrograph of experimentally-induced alveolar tumour. The cells have prominent microvilli, apical cytosomes, and many contain glycogen deposits. From Angus et al, (1985) (16). Reproduced by courtesy of the Editors of Slow viruses in sheep, goats and cattle. CEC Report EUR8076EN, Luxembourg.

In the absence of a specific diagnostic test, objective assessment of the prevalence of SPA within countries and within individual flocks has proved difficult. Earlier reports from Kenya, Britain (23) and South Africa (3) suggested that annual losses attributable to SPA were less than 5%. However, recent assessments from South Africa (24) and Britain (25) show that SPA accounts for almost 70% of all ovine neoplasms and in Britain represents 4.1 diagnoses per 1000 submissions to the Veterinary Investigation Service (25). In Scotland, the disease is even more common, and was detected during a 12 month period in almost 20% of all sheep submitted to the Veterinary Investigation Service (4).

Within affected flocks, Tustin (3) reported that jaagsiekte was responsible for an average annual mortality of 2% (range 0.3% to 24%).

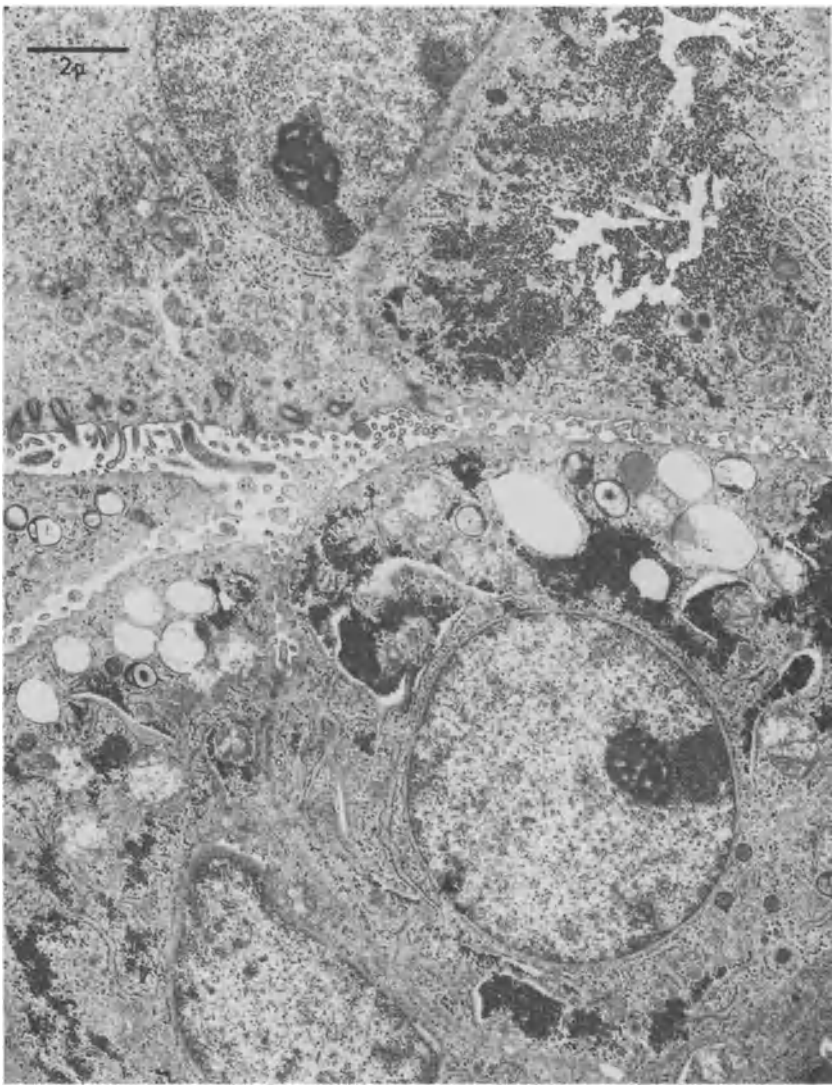

Fig. 15. Higher power of alveolar tumour cell (lower right). Typical features are the apical cytosomes, large glycogen deposits, numerous mitochondria and moderate amounts of endoplasmic reticulin. The centrally-placed nucleus is spherical, with peripheral chromatin. A ciliated cell from the associated bronchiole (upper left) can also be seen.

However, in Scotland, prospective longitudinal studies on two flocks have revealed that SPA, confirmed by histological examination, occurred in 30% of sheep during the commercial life of the flock and was responsible for over 50% of the mortality (Sharp, J.M., Angus, K.W., Scott, F.M.M. and Marinello, P.D., unpublished work).

Breeds and species susceptibility

SPA has been recorded in many breeds of sheep and it is doubtful if any is totally resistant to the disease (3, 11). However, it does appear that some breeds, and even families, may be more resistant than others. Tustin (3) reviewed reports suggesting that breed and familial resistance may occur and that the ram played a major part (26). Experimental studies, in which SPA was induced rapidly in young lambs (6), have revealed that, whilst the incubation period is only 3-6 weeks in most lambs, it is always protracted in those of certain breeds (Sharp, J.M. and Angus, K.W., unpublished work). These observations support the view that breed and/or familial factors may influence susceptibility to SPA.

SPA is a disease of sheep and goats. It has never been observed in other ruminants, nor transmitted experimentally to a wide range of laboratory rodents.

Previous doubts concerning the occurrence of jaagsiekte in goats (3, 11) have been dispelled by the experimental transmission of the disease to young goats (27). Although the efficiency of transmission was much lower in goats and the disease remained subclinical, the histological appearance of the lesions was indistinguishable from that in sheep.

Sex

Jaagsiekte is not restricted to one sex and small numbers in previous reports make it difficult to form an opinion on susceptibility in either sex (26, 28). However, a recent large survey in Peru indicates that male sheep, whether castrated or not, have a higher incidence of the disease (De Martini, J.C., personal communication). Closer examination of the data and husbandry conditions may reveal whether the higher incidence is influenced by sex rather than differences in management of each sex.

Clinical pathology

Aberrations of various parameters have been claimed to occur

during the preclinical stages of SPA (29, 30, 31). The most extensively studied of these has been the concentration of IgG in serum. Nobel et al (31) and Hod et al (29) reported that by comparison with a group not matched for age, sex, management or pregnancy the serum IgG concentration was persistently elevated for several years preceding the onset of clinical signs in sheep with SPA. However, within the same flock, there was no difference between the levels observed in tumour-bearing sheep and sheep without SPA. Similarly Joshi et al (32) reported that elevated concentrations of IgG were not specific for SPA. During a prospective survey of Scottish sheep, we have found that there are no differences in the serum concentrations of IgG, IgM and IgA of sheep with SPA and their unaffected flockmates. There are distinct fluctuations in globulin concentration with the season which can be attributed to the dilution of globulins that accompanies the increased plasma volume during pregnancy (33).

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SHEEP PULMONARY ADENOMATOSIS: STUDIES ON ITS AETIOLOGY

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ABSTRACT

Sheep pulmonary adenomatosis (SPA) is a contagious lung tumour that can be transmitted experimentally. Two viruses have been associated with the disease, a herpesvirus and a retrovirus.

All ovine herpesviruses are related antigenically and have been isolated only from SPA tumour tissues. They do not appear to cause the tumour and their association with SPA appears to arise from reactivation of latent virus.

SPA tumour tissue and lung fluid contain a retrovirus that has properties similar to those of B-type and D-type retroviruses. Homogenates of tumour that contain this retrovirus can transmit SPA to experimentally inoculated sheep. Various retroviruses have been cultured from such tumours. Only one of these has properties similar to that of the retrovirus detected in the tumour and it can transmit SPA experimentally; the others appear to be isolates of the non-oncogenic ovine lentivirus, maedi-visna virus, and play no part in the aetiology of SPA.

INTRODUCTION

Although SPA had been reported earlier to be contagious (reviewed in 1), it was not until a major epizootic occurred in Iceland that this was established clearly, and experimental transmission was demonstrated unequivocally (2). Dungal (2) further showed that the exhaled respirations of affected sheep contained an infectious agent, which was probably viral as it passed through a variety of small pore filters. It was not, however, until Mackay (3) isolated a herpesvirus and Perk *et al*, (4) described a retrovirus in SPA tumour tissue that the existence of such viruses was demonstrated.

This article will review the information that is available on these two viruses, which has led to the current opinion that SPA is caused by a retrovirus, and that the herpesvirus has no aetiological role.

Herpesvirus

A herpesvirus was demonstrated first in Scotland in macrophages cultured from SPA tumour (3). Morphologically similar viruses have since been associated with SPA in Yugoslavia, Kenya and South Africa (5, 6, 7).

Scottish isolates of the SPA herpesvirus have a close antigenic relationship, which is shared with the South African isolates, but no antigenic relationship has been demonstrated with any other herpesvirus, including the taxonomically related bovid herpesviruses (8, 9). Although strains of SPA herpesviruses cannot be differentiated by serological tests, they may be distinguished by restriction endonuclease analysis of their genomic DNA. Within the Scottish isolates, Scott (8) has determined two restriction endonuclease profiles that clearly are different from the South African isolate (Fig. 1). This situation, therefore, is similar to that which occurs with herpesviruses in other species.

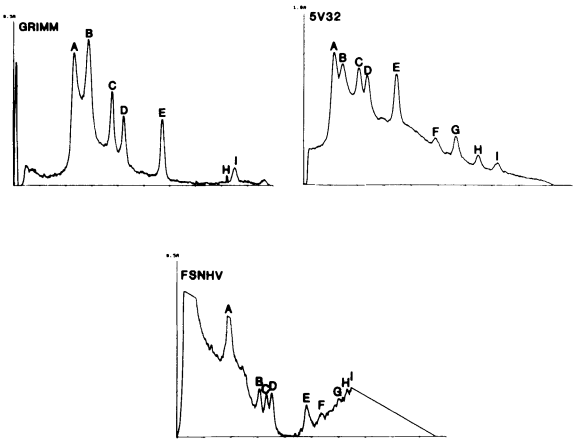

Fig. 1. Densitometer traces of autoradiographs of CHV1 DNA digested with EcoR1 endonuclease. GRIMM and 5V32 isolated in Scotland, FSNHV isolated in South Africa. Reproduced by courtesy of Dr. F.M.M. Scott, Moredun Research Institute.

Thus, these SPA herpesviruses, which are the only herpesviruses to have been isolated from sheep, form a group of serologically indistinguishable viruses that are distinct from all other herpesviruses. In accordance with the recommendations of the Herpesvirus Study Group of the International Committee on Taxonomy of Viruses, the SPA herpesviruses will be referred to in this article as caprine herpesvirus 1 (CHV1) (10).

Isolates of CHV1 have been obtained only from SPA tumours and never from any other tissue from affected sheep nor the lungs nor alveolar macrophages of unaffected sheep (11). This strong positive correlation between CHV1 and SPA suggested a possible involvement of this virus in the aetiology of the disease. However, numerous attempts to reproduce SPA experimentally by the inoculation of sheep with CHV1 have failed (7, 8, 11). This conundrum regarding the role of CHV1 has plagued SPA research for many years. The position was confused further by a report suggesting that combined infection with CHV1 and the SPA retrovirus resulted in increased tumour development compared with the retrovirus alone (12). Recently, however, the role of CHV1 has become clearer as a result of combined studies on its epidemiology and pathogenesis.

It was reasoned that if there were a direct relationship between SPA and infection with CHV1 then two consequences would obtain. First, most cases of SPA would have antibodies to CHV1 and sheep without SPA would have no antibodies, or lower titres. Secondly, in countries where the tumour does not occur, the prevalence of infection by CHV1 would be low or absent. In both South Africa and Scotland, it was found that infection by CHV1 is widespread, with no difference in titre or proportion of infected animals between sheep with SPA and those without (8, 13). Similarly, antibodies to CHV1 are widespread in countries where SPA is absent (8). These studies established that infection of sheep by CHV1 is distributed globally and is not necessarily associated with the occurrence of SPA. Although these epidemiological findings are in agreement with the view from the earlier transmission studies that CHV1 is not involved in the aetiology of SPA, they provide no clue as to why the virus has been isolated only from SPA tumour material. A solution to this question has been provided by more recent investigations on the pathogenesis of CHV1.

Previous studies on the pathogenesis of CHV1 have been long-term and focussed on the hypothetical ability of the virus to induce adenomatous changes, overlooking the acute stages of infection and sites of virus replication. Recent experiments have shown that infection of young lambs with CHV1 results in a subclinical interstitial pneumonia (7, 8, 14).

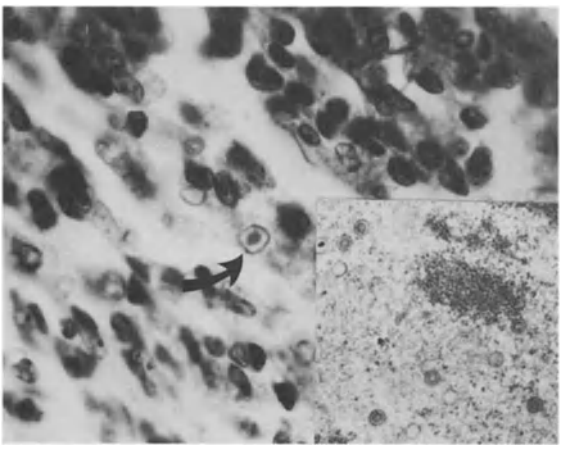

Fig. 2. Interstitial pneumonia in lung of lamb inoculated with CHV1. Intranuclear inclusion (arrowed) present in histiocyte. Inset: herpesvirus particles within nucleus of alveolar macrophage. From F.M.M. Scott et al (1984) (14). Reproduced by courtesy of the Editors, Archives of Virology (Springer-Verlag, Wien/New York).

The virus replicates mainly in the respiratory tract and the demonstration of intranuclear inclusion bodies and virions only in pulmonary histiocytes suggests that the main site of replication is the macrophage (14) (Fig. 2). In common with other herpesviruses, CHV1 can establish a latent infection within the respiratory tract (8, 14). Reactivation of this virus, therefore, offers an attractive explanation for the isolation of CHV1 only from adenomatous tissue. Failure to isolate CHV1 from other diseases and the reinforcement of its association with SPA probably arises from the strict requirement for virus replication only in macrophages and discrimination against isolation of CHV1 by other more standard isolation procedures employed in diagnostic laboratories.

Retrovirus

In the early 1970's, virus particles, morphologically similar to retrovirus, were detected in SPA tumours by electron microscopy (4, 15). Two different forms of particle were described, which corresponded with the type A and type C particles of retroviruses (Fig. 3). There is now general agreement that at least one of these forms, the type A particle, may be found in the epithelial tumour cells (15, 16) or budding from the plasma membrane of natural cases of SPA (Sharp, J.M. Angus, K.W. and Gray, E.W., unpublished observation).

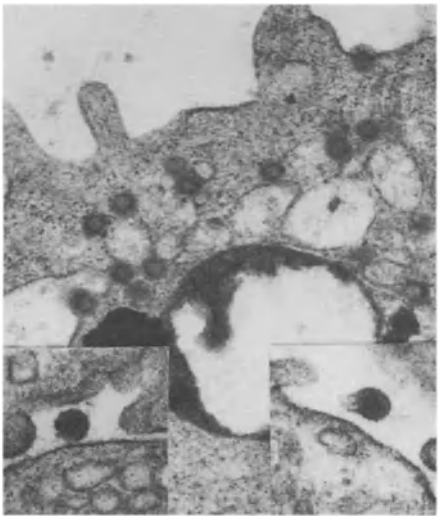

Fig. 3. Clusters of intracytoplasmic type A particles in epithelial tumour cells. Insets: extracellular particles.

The association of a retrovirus with SPA was strengthened further by biochemical investigations demonstrating reverse transcriptase activity associated with a particle with the buoyant density of a retrovirus (12, 17). Although neither of these reports could be regarded as conclusive, subsequent studies have provided clear evidence for the presence within SPA tumour and lung fluids of a retrovirus (18, 19). Reverse transcriptase activity was shown to have a preference for Mg^{2+} and was associated with a particle with a density in sucrose of 1.18 $g.ml^{-1}$. Taken together with the demonstration of intracytoplasmic and budding type A particles, these biochemical properties suggested that the SPA retrovirus might be more akin to the type B and type D retroviruses rather than type C (18).

The proposed relationship to type B and, particularly, type D retroviruses was borne out by the demonstration of an antigenic relationship between a 25,000 mw protein (p25) in SPA tumours and lung fluids and the major internal proteins of mouse mammary tumour virus and Mason-Pfizer monkey virus, the prototype type B and type D retroviruses (20). Both the p25 protein and reverse transcriptase activity can be detected in sucrose gradients at a density of 1.18 $g.ml^{-1}$ and, therefore, p25 appears to be a structural protein in the SPA retrovirus (Fig. 4).

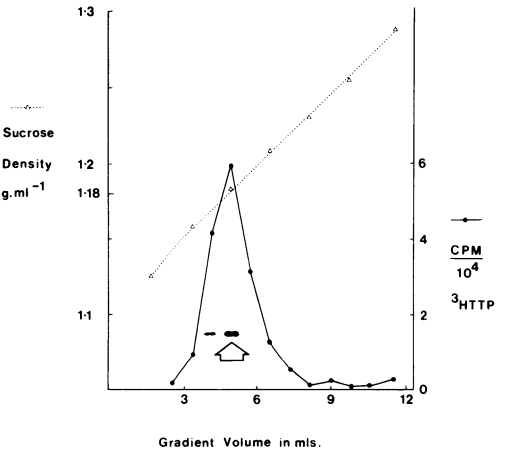

Fig. 4. Demonstration of reverse transcriptase activity (●——●) and p25 (arrow) in SPA retrovirus that is sedimenting at a density of 1.18 $g.ml^{-1}$ on a sucrose gradient.

A protein of the same size and antigenic cross-reactivity has since been demonstrated in SPA lung fluids or tumours from Scotland, South Africa and Peru (21), Northern Ireland (22), Israel (Sharp, J.M. and Perk, K., unpublished) and the U.S.A. (Sharp, J.M., DeMartini, J.C., Rosadio, R. and Russell, H., unpublished). It is clear therefore that these retroviruses, which are present in SPA, possess a common antigen on their p25 that, taken with their similar biochemical properties, indicates that they are probably the same virus.

It has been known for several years that homogenates of tumour can be used to transmit SPA experimentally (2, 23). Therefore, following the demonstration of retrovirus in SPA tumours, the induction of SPA in

sheep by inoculation of sucrose gradient fractions from tumour containing retrovirus suggested a role for this virus in the aetiology of SPA (12, 18, 19). No virus particles were observed either in the induced tumours or cell cultures derived from these, therefore direct evidence of virus replication was lacking. However, indirect evidence was obtained by the demonstration of particle-associated reverse transcriptase activity in tumour and lung fluid, although not in cell cultures.

Further advances in elucidating the structure of the retrovirus arose from the development of a means of inducing SPA in newborn lambs (9, 24). The incubation period of the disease was reduced to 3-6 weeks but, more importantly, many virus particles were observed (24). Groups of intracytoplasmic and, occasionally, budding type A particles were seen only in the epithelial tumour cells and, in close proximity to these cells, many extracellular virions with the morphology of type B and type D retroviruses (24) (Fig. 5).

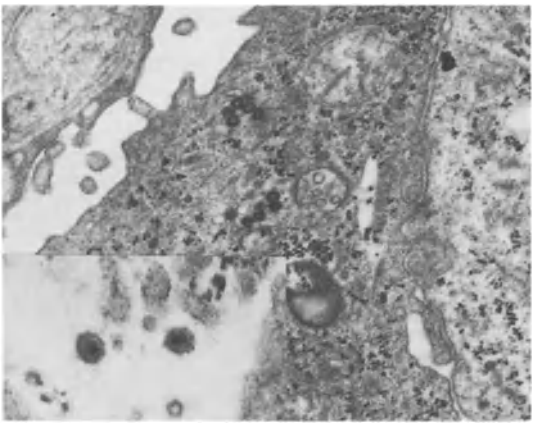

Fig. 5. Lung of lamb 23 days post-inoculation. Clusters of intracytoplasmic type A particles in epithelial tumour cells. Inset: extracellular particles showing cylindrical core and short envelope projections. From J.M. Sharp et al (1983) (24). Reproduced by courtesy of the Editors, Archives of Virology (Springer-Verlag, Wien/New York).

Thus, these observations concur with the earlier biochemical and antigenic findings (18, 20) that the retrovirus associated with SPA resembles type B and type D retroviruses.

These findings to date have provided reasonable grounds for the hypothesis that the retrovirus described above is involved in the aetiology of SPA. A major obstacle to the acceptance of this view has

been that, as a result of the failure to develop in vitro culture systems for the SPA retrovirus, all inocula for transmission experiments have been derived directly from tumour or lung fluid. Consequently, it was difficult to exclude the involvement of other adventitious agents.

However, it has been reported recently that replication of the SPA retrovirus, as determined by the presence of reverse transcriptase activity and p25, occurred in cell cultures derived from tumours induced in young lambs (24, 25). Replication appeared to continue for up to 96 days, although it was barely detectable in the later stages. Concentrated supernates from these cell cultures were able to induce SPA in one of 3 lambs inoculated intratracheally within 24 hours of birth (Fig. 6) (Sharp, J.M., Angus, K.W., Jassim, F.A. and Gray, E.W., unpublished observations). These experiments, therefore, have fulfilled Koch's postulates and provided clear evidence that the SPA retrovirus, which is related to type B and type D retroviruses, is involved in the pathogenesis of SPA.

Other retroviruses have been isolated from SPA tumour and propagated in vitro (26, 27). These viruses are, however, unlikely to play any part in the aetiology of SPA because their morphological, biochemical and antigenic properties distinguish them as members of the non-oncogenic ovine lentivirus sub-family.

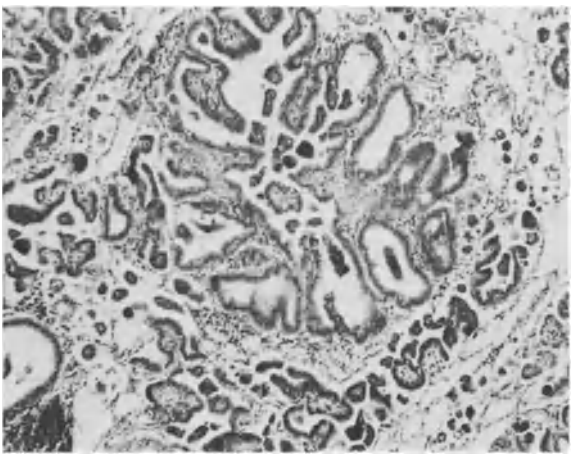

Fig. 6. Typical adenomatous lesions in the lungs of a lamb inoculated with SPA retrovirus grown in cell cultures.

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