Onderstepoort J. vet. Res., 50, 317–322 (1983)

THE MORPHOLOGY AND MORPHOGENESIS OF JAAGSIEKTE RETROVIRUS (JSRV)

ANNA-LISE PAYNE[1], D. W. VERWOERD[1] and HELEN M. GARNETT[2]

ABSTRACT

PAYNE, ANNA-LISE, VERWOERD, D. W. & GARNETT HELEN, M., 1983. The morphology and morphogenesis of jaagsiekte retrovirus (JSRV). *Onderstepoort Journal of Veterinary Research*, 50, 317–322 (1983).

Jaagsiekte retrovirus (JSRV) was recently shown to be the aetiological agent of jaagsiekte (ovine pulmonary adenomatosis). The morphogenesis of JSRV was studied in jaagsiekte tumour tissue. Intracytoplasmic particles, often associated with centrioles, were found in tumour cells. JSRV budded from tumour cells with a complete core which appeared to mature during the budding process. Extracellular particles were found in the alveolar lumen. Immature extracellular particles were rare. Mature extracellular JSRV was membrane-bound and had a slightly eccentric nucleoid with an electron-dense perinucleoidal space. In negatively stained preparations of JSRV the envelope was covered with spikes. JSRV is morphologically distinct from all known retroviruses.

INTRODUCTION

Jaagsiekte or pulmonary adenomatosis is a transmissible contagious tumour affecting the lungs of sheep. Recent experimental evidence indicates that a retrovirus is the aetiological agent of the disease (Verwoerd, Williamson & De Villiers, 1980; Verwoerd & Williamson, 1982). Jaagsiekte retrovirus (JSRV) can be isolated from lung washes, and electron microscopy of the virus revealed membrane-bound, electron-dense particles (Verwoerd & Williamson, 1982). The purification, biochemical and serological properties of JSRV are discussed in an accompanying paper (Verwoerd, Payne, York & Myer, 1983).

Owing to a lack of an *in vitro* system for culturing JSRV the morphogenesis of the virus was studied in jaagsiekte tumour tissue. As JSRV possesses an RNA-dependant DNA polymerase and induces tumours it is most likely to be related to members of the subfamily *Oncovirinae* of the family *Retroviridae* (Matthews, 1982). Therefore, the morphology of JSRV was compared with members of the 3 genera making up the *Oncovirinae*. These included mouse mammary tumour virus (MMTV) a type B oncovirus, murine sarcoma virus (MuSV), a type C oncovirus, and squirrel monkey retrovirus (SMRV), a type D oncovirus. Besides JSRV there are a number of lentiviruses that are known to infect sheep (Weiss, Sweet, Gulati & Harter, 1976) as well as ovine leukaemia virus (Rhode, Pauli, Paulsen, Harms & Bauer, 1978). Maedi-Visna virus (MVV) is the prototype of the subfamily *Lentivirinae*. Ovine leukaemia virus is closely related to, if not identical with, bovine leukaemia virus (BLV), an unclassified oncovirus (Rhode *et al.*, 1978). The morphology and morphogenesis of both viruses were compared with those of JSRV. A comparison of JSRV with retrovirus-like particles found in jaagsiekte lungs in Israel (Perk, Hod & Nobel, 1971; Hod, Perk, Nobel & Klopfer, 1972; Hod, Herz & Zimber, 1977) was also made.

MATERIALS AND METHODS

Abbreviations used: JSRV—jaagsiekte retrovirus. BLV—bovine leukaemia virus. MMTV—mouse mammary tumour virus. MuSV—murine sarcoma virus. MVV—Maedi-Visna virus. SMRV—squirrel monkey retrovirus. MPMV—Mason-Pfizer monkey virus. GA—glutaraldehyde.

Animals

Lung tissue samples, obtained from 50 sheep suffering from experimentally induced jaagsiekte, were fixed in 2,5 % gluteraldehyde in 0,1 M cacodylate buffer with 4 % sucrose, pH 7,2 (GA fixative) for electron microscopy. Ten lung samples from normal sheep were included as negative controls.

[1] Veterinary Research Institute, Onderstepoort 0110

[2] Department of Microbiology, University of the Witwatersrand, Johannesburg 2000

Received 9 September 1983—Editor

Cell cultures

The origins of the cell cultures used for the production of MuSV, BLV, MMTV and SMRV have been described in an accompanying paper (Verwoerd *et al.*, 1983). Standard techniques were used for their culture, and virus production was estimated by means of a reverse transcriptase assay (Verwoerd *et al.*, 1980). Maedi-Visna virus (ZZV 1050) was kindly donated by Dr D. J. Houwers, Lelystad, Netherlands. The virus was propagated in sheep choroid plexus cells and handled in our foot-and-mouth containment laboratory. Monolayers were removed with a rubber policeman, suspended in GA fixative, placed in microhaematocrit tubes and pelleted using a Damon IEC MB centrifuge.

Virus

JSRV, BLV, MMTV, MuSV, SMRV and MVV were purified as outlined in the accompanying paper (Verwoerd *et al.*, 1983).

Thin section electron microscopy

Lung samples and cell culture pellets were fixed for a minimum of 1 hour in GA fixative, postfixed for 1 hour in 1 % osmium tetroxide in 0,1 M cacodylate buffer containing 4 % glucose, pH 7,2. After dehydration in a graded acetone series the samples were cleared in propylene oxide and embedded in Epon. Thin sections were stained with 2 % aqueous uranyl acetate and lead citrate (Reynolds, 1963) and examined in a Siemens 102 electron microscope.

Negative stain electron microscopy

Formvar-carbon-coated grids were floated consecutively on drops of virus suspension, 1 % osmium tetroxide, distilled water and 3 % phosphotungstic acid, pH 6,0. The time on each droplet varied between 10 seconds and 1 minute. The osmium tetroxide step was omitted with MMTV and MuSV.

Calibration of the electron microscope

To measure the size of JSRV accurately, Fullham chromium replica grating suspension was used as an internal calibration standard.

RESULTS

Jaagsiekte lungs

A study was made of the ultrastructure of the lesions to identify the cell types with which the virus particles were associated (Payne & Verwoerd, 1984). Virions were found in 11 of the 50 jaagsiekte lungs and in none of the control lungs examined. In 7 of the lungs intracytoplasmic particles were found within the tumour cells. Extracellular particles were found in 7 jaagsiekte lungs, indicating that only 3 lungs contained both types of particle.

The intracytoplasmic virions had a mean diameter of 74 nm and were usually found as single particles or in clusters of 2 or 3 particles (Fig. 1). Some virions were found in the vicinity of centrioles (Fig. 2). Most intracytoplasmic particles had a small, electron-lucent centre surrounded by 2 shells. The inner shell was more electron-dense than the outer shell, which was sometimes covered with projections (Fig. 1).

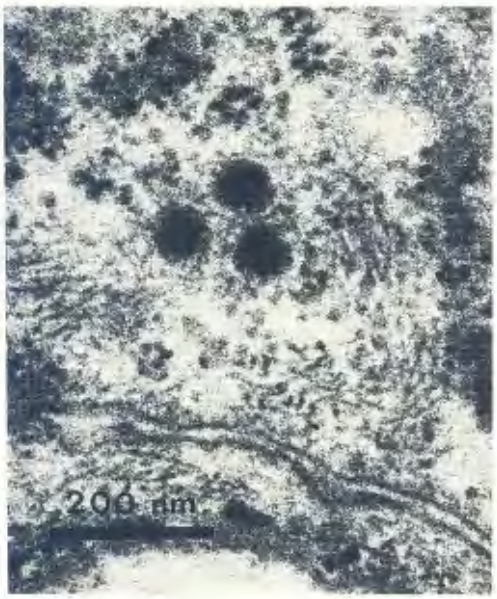

FIG. 1 A cluster of 3 intracytoplasmic virus particles in a jaagsiekte tumour cell

A more condensed and uniformly electron-dense form of intracytoplasmic particle was found usually at the tips of tumour cell microvilli (Fig. 3). In thin sections these particles did not have surface projections and were interpreted as representing the 1st stages in the budding of JSRV. Particles in the final stages of budding were also found at the tips of microvilli (Fig. 4). Immature virions were rare and the virus appeared to mature during the budding process. Single particles were also observed budding into small vacuoles within the tumour cells.

Extracellular particles were usually found in the alveolar lumen. These particles were identical with those observed in thin sections of virus purified from jaagsiekte lung washes (Verwoerd *et al.*, 1980; Verwoerd & Williamson, 1982). The purified virus was shown to induce jaagsiekte experimentally (Verwoerd & Williamson, 1982). On rare occasions virus particles were observed in the inter-tumour cell spaces as well as tumour cell vacuoles. Extracellular particles of JSRV had a mean diameter of 121 nm. Mature JSRV had a unit membrane and a slightly eccentric nucleoid with an electron-dense perinucleoidal space (Fig. 5).

Negatively stained JSRV

Fixed, negatively stained preparations of JSRV revealed that the envelope was covered with spikes (Fig. 6). Negatively stained JSRV had a mean diameter of 107 nm and the spikes were 10–12 nm in length with an interspike distance of 11–13 nm. Each spike consisted of a knob on a narrow spine. Many of the fixed, negatively stained JSRV particles were attached to a small bleb of smooth membrane. Preparations of JSRV that were not fixed prior to negative staining appeared to be unstable and most of the virus disintegrated. The remaining virus appeared flattened in a tail and head configuration.

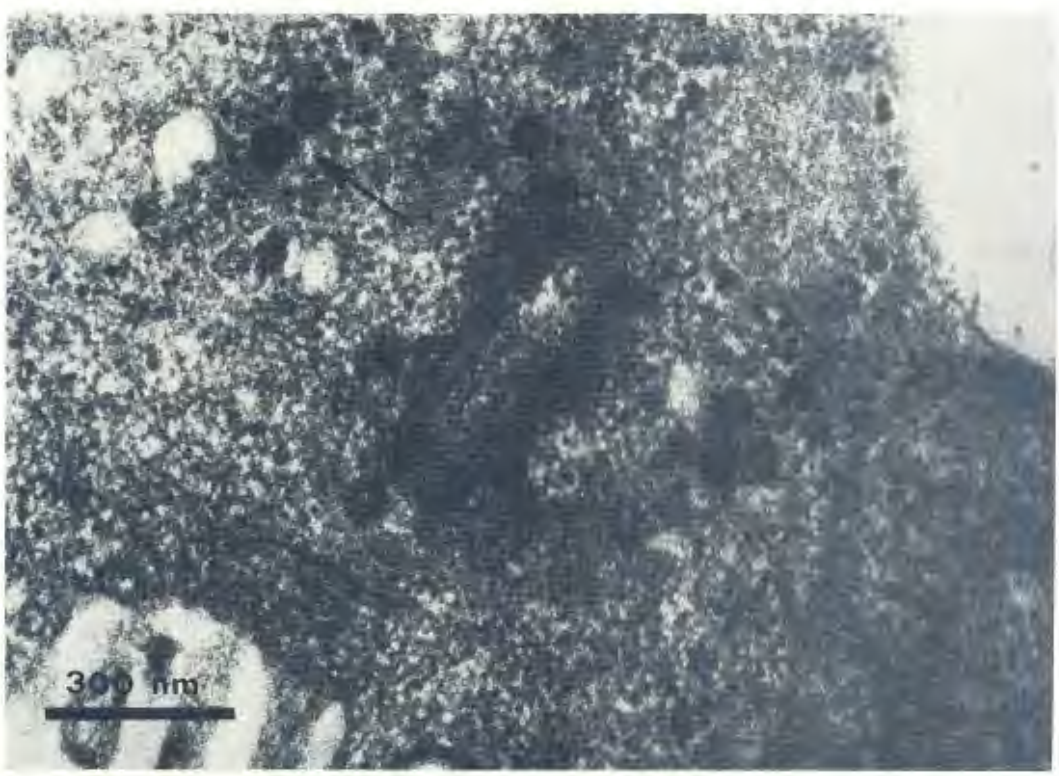

FIG. 2 Intracytoplasmic particles (arrows) found in the vicinity of a centriole in a jaagsiekte tumour cell

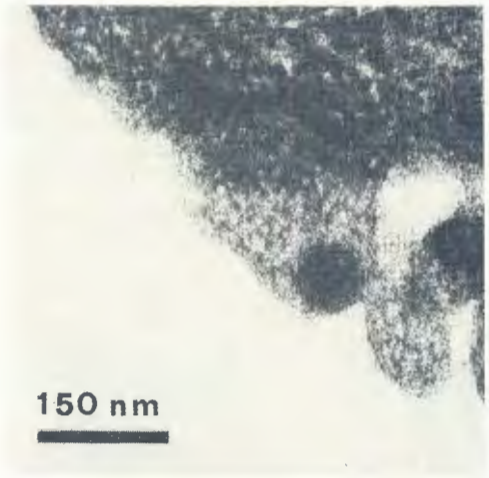

FIG. 3 Virus particle in the 1st stage of budding from a microvillus of a jaagsiekte tumour cell

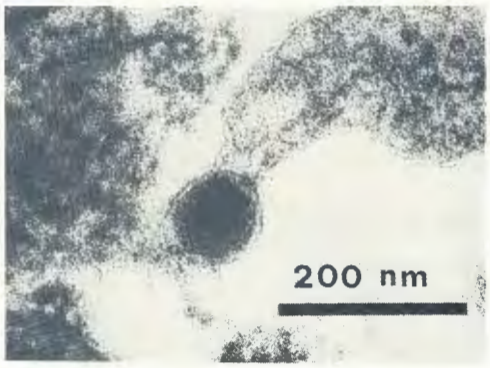

FIG. 4 Virus particle in the final stages of budding from a microvillus of a jaagsiekte tumour cell

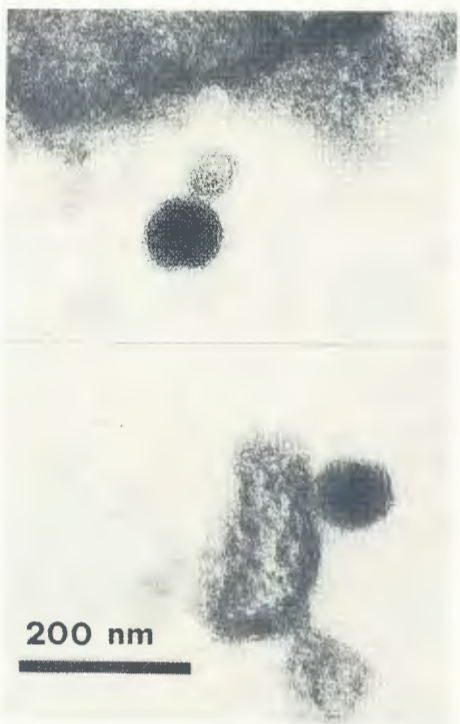

FIG. 5 Mature extracellular JSRV particles (arrows) in the alveolar lumen of a jaagsiekte lung

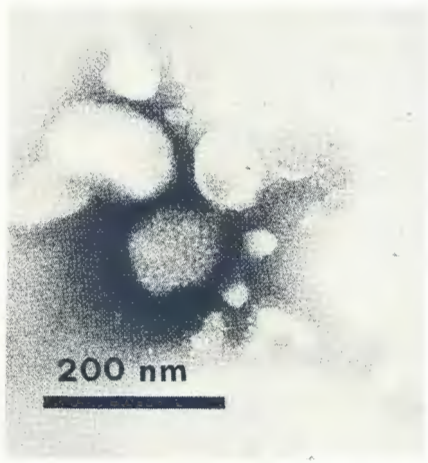

FIG. 6 JSRV negatively stained with PTA after fixation in osmium tetroxide. Note the spikes in the membrane

Comparison of JSRV with four RNA tumour viruses (Fig. 7)

The ultrastructure of JSRV was compared with those of BLV, MMTV, MuSV, and SMRV. Intracytoplasmic particles were found in JSRV, MMTV and SMRV. However, the quantity of intracytoplasmic JSRV particles found in jaagsiekte tumours was far less than that of MMTV in mouse tumours. With JSRV it was rare to find more than 4 particles whereas with MMTV clusters of over 50 particles were often seen. The A particles of MMTV and SMRV consist of 2 rings, the inner ring being more electron-dense than the outer ring. Some of the JSRV intracytoplasmic particles appeared to have this double ring structure, but the electron-lucent centre was either much smaller than that observed in MMTV and SMRV, or absent. The projections observed on the outer shell were not oberserved in MMTV or SMRV. However, similar projections have been observed on MPMV A particles (Schidlovsky, 1977). BLV and MuSV have no intracytoplasmic form of the virus.

The intracytoplasmic particles of JSRV appeared to undergo some form of maturation while budding. That only 1 immature particle was observed in jaagsiekte tumour tissue indicates that this form of budding is extremely rare, unlike that in SMRV, MuSV and MMTV where it is considered the norm. Immature particles were also rare in bat cells producing BLV. As with those of JSRV, BLV particles with an electron-dense nucleoid were seen still attached to the membrane, suggesting that maturation took place while the particles were budding. This confirms the findings of Calafat & Ressang (1977). MuSV buds with a typical C type, crescent-shaped nucleoid to form an immature particle. This is completely different from the budding morphology of JSRV. Budding of JSRV and MMTV often took place at the tips of microvilli. In all the RNA tumour viruses studied, virus particles were observed within cytoplasmic vacuoles.

The nucleoid of JSRV was slightly eccentric, while the nucleoids of BLV, MuSV and SMRV are centric and those of MMTV are eccentric. The perinucleoidal space is relatively electron-lucent in BLV, MMTV, MuSV and SMRV and electron-dense in JSRV. An electron-dense perinucleoidal space has been observed in guinea-pig retrovirus (Schidlovsky, 1977) and in retrovirus-like particles observed in the placenta of baboons (Panem, 1979).

Of the RNA tumour viruses studied with the use of negative staining techniques, only MMTV had spikes similar to those of JSRV. The length of the spikes on MMTV is 9–8 nm and the interspike distance 4,8 nm (Sarkar & Moore, 1970). JSRV spikes were slightly longer (10–12 nm), with a greater interspike distance (11–12 nm).

Comparison of JSRV with Maedi-Visna virus (Fig. 7)

No intracytoplasmic forms of MVV were observed in this study. However, intracytoplasmic, multilayered spherical structures have been observed in some cell lines infected with MVV (Coward, Harter, Hsu & Morgan, 1972). These structures do not resemble JSRV.

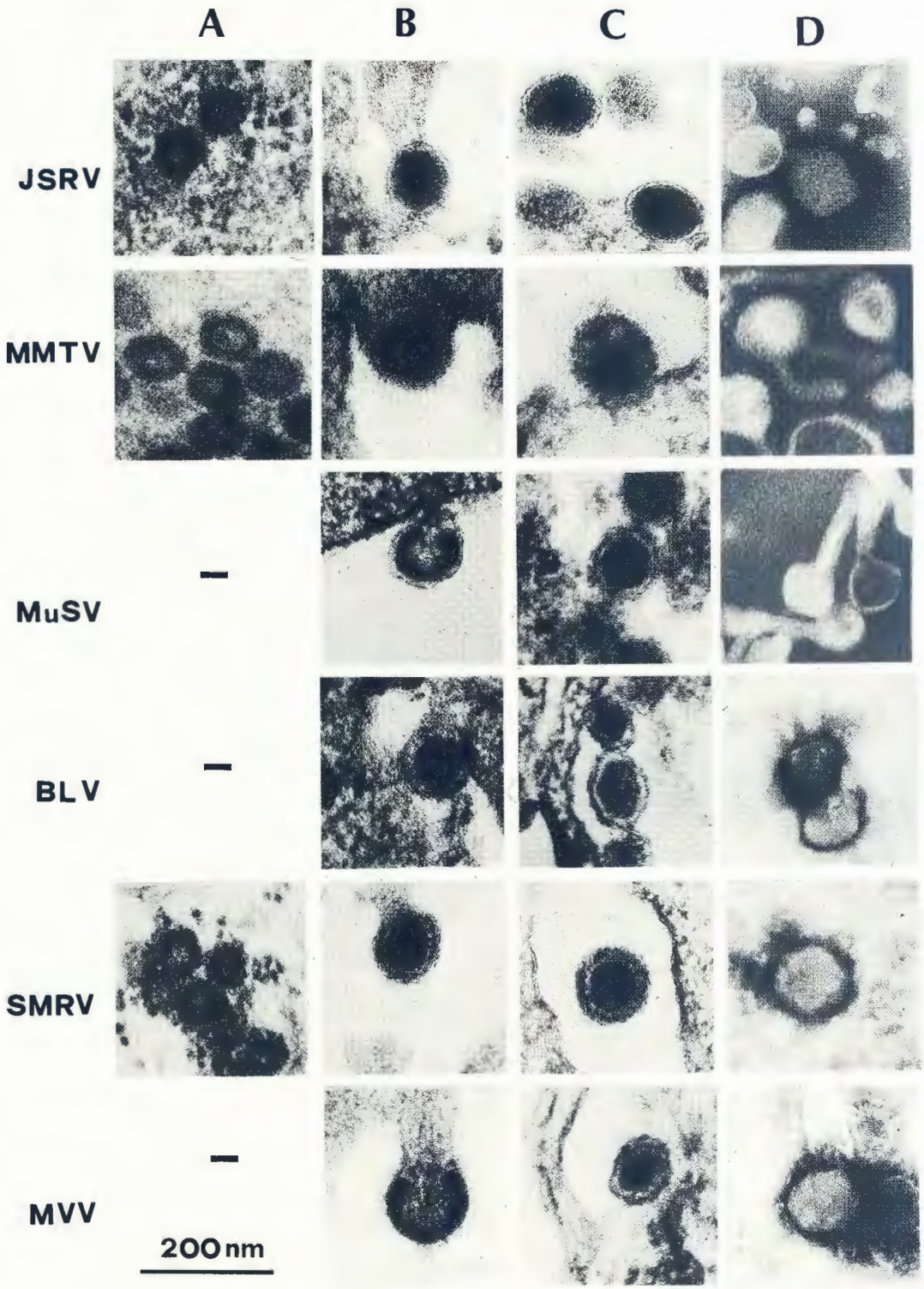

FIG. 7 A comparison of intracytoplasmic (A), budding (B), mature (C) and negatively stained (D) JSRV with the same preparations of MMTV, MuSV, BLV, SMRV and MVV. MuSV, BLV and MVV do not usually have the intracytoplasmic stage of morphogenesis

MVV budded with a crescent-shaped nucleoid to form immature particles with the nucleoid immediately below the viral membrane, and had no perinucleoidal space. The budding and immature particles of MVV were very distinctive, and did not resemble JSRV. Extracellular mature MVV had a centric ovoid or conical core with a clear intermediate layer and an electron-lucent perinucleoidal space. Negatively stained MVV lacked surface structure in this study. However, Haase & Baringer (1974) published a micrograph of negatively stained Visna virus with surface projections. These particles do not resemble negatively stained JSRV.

Discussion

Intracytoplasmic JSRV particles were found in tumour cells on the periphery of the lesion, usually towards the apex of the cell. The extracellular JSRV particles were usually found in close proximity to the tumour cells in the alveolar lumen. These particles were seen budding from tumour cells and this indicates that the virus replicates in these cells. Only 22 % of the lungs examined contained intracytoplasmic or extracellular forms of the virus. Most of the lungs studied were rinsed prior to sampling for transmission electron microscopy. This procedure removes a large amount of the extracellular JSRV present in the lungs and probably explains the low percentage of lungs found to contain extracellular virus when electron microscopy techniques were employed. It is of interest to note that all jaagsiekte lung washes contained JSRV, as demonstrated by the RNA-dependent DNA polymerase assay (Verwoerd *et al.*, 1983). However, this does not explain the low percentage of lungs containing intracytoplasmic and budding JSRV. It could be that the tumour cells all contain the provirus sequences necessary for transformation but that only a few cells were actively producing JSRV.

It is difficult to determine the morphogenesis of a virus when its replication cannot be synchronized in cell cultures and when it is present in low quantities in host tumour tissue. The relationship between the intracellular particles and the extracellular particles is uncertain. It is possible that the particles are assembled deep within the cytoplasm and then migrate to the plasma membrane where they bud to form mature extracellular particles. The close proximity of some of the intracellular particles to the centrioles suggests a role for the microtubules in the transport of the intracellular particles to the surface of the cell. The association of intracytoplasmic particles with the mitotic apparatus is not unique to JSRV. The South-east Asian mouse retrovirus isolates M432 and M832 are very similar to those of JSRV both in the morphology of their intracytoplasmic particles and in the association of these particles with the mitotic apparatus (Heine & Todaro, 1978). These authors suggest that microtubules may play a role in transportation of viral precursors to the cell membrane. Indeed, microtubules are known to play a role in the transport of intracellular endogenous virus particles in Chinese hamster cells (Heine, Kramarsky, Wendel & Suskind, 1979). Once again the intracytoplasmic particles in these cells are closely associated with the sites of mirotubule formation. Microtubules are also known to play a role in the assembly of intracellular particles. Satake & Luftig (1982) suggest that cytoplasmic microtubules may play a role in recruiting murine leukaemia virus p65 from its site of synthesis to the plasma membrane.

There is a change in morphology from the intracytoplasmic particles deep within the cytoplasm to those at the plasma membrane. This indicates that the particles may undergo some form of maturation before budding, although the possiblity that the particles are not related cannot be excluded. No budding particles were observed with incomplete cores. The majority of JSRV budded with condensed cores to form mature particles. As the particles observed in the late stages of budding did not differ significantly from extracellular virus or purified virus (Verwoerd *et al.*, 1980; Verwoerd & Williamson, 1982), we concluded that maturation of the JSRV core took place before and during the budding process.

The ultrastructure and morphogenesis of JSRV differ from those observed in the other retroviruses studied. It should be noted that JSRV morphogenesis was studied in tumour tissue while the other retroviruses were studied in cell cultures. As in JSRV the cores of MMTV and SMRV are assembled intracytoplasmically. However, the intracytoplasmic particles of JSRV differ morphologically from those of SMRV and MMTV and resemble those of South-east Asian retrovirus isolates M432 and M832 (Heine & Todaro, 1978). M432 and M832 are similar but not identical with MMTV, and are regarded as new members of the type B oncoviruses. The main differences between MMTV and these viruses are the position of the nucleoid, which is centric in M432 and M832, and the ultrastructure of the intracytoplasmic particles (Heine & Todaro, 1978). JSRV, MMTV and sometimes BLV bud from the plasma membrane with complete cores. On the other hand, cores of MuSV and MVV are assembled at the cell membrane and are incomplete during budding. This form of budding is characteristic of all type C viruses and lentiviruses, implying that JSRV is not related to either of these groups of viruses.

Dalton (1972) regarded extracellular retroviruses with condensed cores as being mature, whereas "enveloped A particles" are regarded as immature particles. Only JSRV and BLV bud to form mature particles, and indication of maturation before and during the budding process. In both these viruses immature particles are extremely rare. MuSV, MMTV, SMRV and MVV bud to form immature particles and maturation of the core takes place extracellularly.

An important difference between mature JSRV and the 5 retroviruses studied is the electron density of the perinucleoidal space. JSRV has a relatively electron-dense, perinucleoidal space when compared with MMTV, MuSV, SMRV, BLV and MVV. Type C virus-like particles found in primate placenta are electron dense below the viral envelope (Panem, 1979). This was interpreted as being a virus with a closely applied envelope and no perinucleoidal space. This is not the case with JSRV, where an outline of the core shell can be seen enclosing the nucleoid, and there is definitely a perinucleoidal space. The position of the nucleoid in JSRV, being slightly eccentric, differs from the other retroviruses studied. As far as the surface structure is concerned, only MMTV has surface spikes resembling those seen on JSRV. However, the spikes on MMTV are shorter and closer together than those of JSRV.

A number of papers have been published on the presence of retrovirus-like particles in the lungs of natural jaagsiekte cases (Perk *et al.*, 1971; Hod *et al.*, 1972; Hod *et al.*, 1977) and jaagsiekte lung homogenates with reverse transcriptase activity (Perk, Michalides, Spiegelman & Schlom, 1974). These studies were done mainly on Israeli Awassi sheep. Intracytoplasmic particles were found in Israeli sheep tumour cells which were similar to MMTV (Perk *et al.*, 1971; Hod *et al.*, 1977). From the published micrographs it would appear that JSRV has a smaller electron-dense centre than the Israeli virus. However, it is difficult to make a proper comparison with JSRV without examining more particles. As with JSRV the intracytoplasmic particles in the Israeli study were seen budding from the microvilli of the tumour

cells (Hod *et al.*, 1977). However, newly budded virus was immature in contrast to the mature virus of JSRV. Perk *et al.*, (1971) described the extracellular particles as being typical type C particles with a centric nucleoid and a relatively electron-lucent perinucleoidal space. These particles are very different from the JSRV particles observed in South African sheep. Type C particles were observed budding from fibroblast-like cells (Hod *et al.*, 1977) and plasma-like cells (Hod *et al.*, 1972) in Israeli jaagsiekte tumour tissue, but none were observed in the South African jaagsiekte lungs studied. The role of the type C virus-like particles in the Israeli sheep suffering from jaagsiekte is unknown. They may represent a virus related to JSRV that causes jaagsiekte in Israel, or they may be a latent virus that appears when the animal is under stress. It is also possible that some of the virions in the Israeli sheep lungs are related to Maedi-Visna virus as this disease is present in Israel but not in South Africa. Whatever their role, these particles are morphologically distinct from the virions seen in South African sheep with experimentally induced jaagsiekte.

JSRV is therefore morphologically distinct from all other retroviruses examined and probably belongs to a new genus within the family of *Retroviridae*. This is confirmed by the results obtained from the biochemical and serological characterization of the virus (Verwoerd *et al.*, 1983).

ACKNOWLEDGEMENTS

We should like to thank Miss J. Hoogenout for technical assistance and Dr P. J. Houwers, Centraal Diergeneeskundig Instituut, Lelystad, Netherlands for providing the Maedi-Visna virus.

REFERENCES

CALAFAT, J. & RESSANG, A., 1977. Morphogenesis of bovine leukemia virus. *Virology*, 80, 42–53.

COWARD, J. E., HARTER, D. H., HSU, K. C., & MORGAN, C., 1972. Ferritin conjugated antibody labeling of Visna virus. *Virology*, 50, 925–930.

DALTON, A. J., 1972. RNA tumour viruses—terminology and ultrastructural aspects of virion morphology and replication. *Journal of the National Cancer Institute*, 49, 323–325.

HAASE, A. T. & BARINGER, J. R., 1974. The structural polypeptides of RNA slow virus. *Virology*, 57, 238–250.

HEINE, U. I. & TODARO, G. J., 1978. New type B retrovirus associated with kinetochores and centrioles of the host cell. *Journal of General Virology*, 39, 41–52.

HEINE, U. I., KRAMARSKY, B., WENDEL, E. & SUSKIND, R. G., 1979. Enhanced proliferation of endogenous virus in Chinese hamster cells associated with microtubules and the mitotic apparatus of the host cell. *Journal of General Virology*, 44, 45–55.

HOD, I., PERK, K., NOBEL, T. A. & KLOPFER, V., 1972. Lung carcinoma of sheep (jaagsiekte). III. Lymph node, blood and immunoglobulin. *Journal of the National Cancer Institute*, 48, 487–507.

HOD, I., HERZ, A. & ZIMBER, A., 1977. Pulmonary carcinoma (jaagsiekte) of sheep. Ultrastructural study of early and advanced tumour lesions. *American Journal of Pathology*, 86, 545–558.

MATTHEWS, R. E. F., 1982. Classification and nomenclature of viruses. *Intervirology*, 17, 124–189.

PANEM, S., 1979. C-type virus expression in the placenta. *Current Topics in Pathology*, 66,175–189.

PAYNE, ANNA-LIŠE & VERWOERD, D. W., 1984. A scanning and transmission electron microscopy study of jaagsiekte lesions. *Onderstepoort Journal of Veterinary Research*, 1984 (in press).

PERK, K., HOD, I. & NOBEL, T. A., 1971. Pulmonary adenomatosis of sheep (jaagsiekte). I. Ultrastructure of the tumour. *Journal of the National Cancer Institute*, 46, 525–537.

PERK, K., MICHALIDES, R., SPIEGELMAN, S. & SCHLOM, J., 1974. Biochemical and morphologic evidence for the presence of an RNA tumour virus in pulmonary carcinoma of sheep (jaagsiekte). *Journal of the National Cancer Institute*, 53, 131–135.

REYNOLDS, E. S., 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *Journal of Cell Biology*, 17, 208–212.

RHODE, W., PAULI, G., PAULSEN, J., HARMS, E. & BAUER, H., 1978. Bovine and ovine leukaemia viruses. I. Characterization of viral antigens. *Journal of Virology*, 26, 159–164.

SARKAR, N. H. & MOORE, D. H., 1970. External structure of mouse mammary tumour virus. *Proceedings of 7th International Electron Microscopy Conference, Grenoble*, 3, 952.

SATAKE, M., & LUFTIG, R. B., 1982. Microtubule-depolymerizing agents inhibit Moloney murine leukaemia virus production. *Journal of General Virology*, 58, 339–349.

SCHIDLOVSKY, G., 1977. Structure of RNA tumour viruses. *In:* GALLO, R. C. (ed.). Recent advances in cancer research: Cell biology, molecular biology and tumour virology, Volume 1. 189–205. Cleveland: CRC Press.

STONE, L. B., SCOLNICK, E., TAKEMOTO, K. K. & AARONSON, S. A., 1971. Visna virus, a slow virus with an RNA dependent DNA polymerase. *Nature*, 229, 257–258.

VERWOERD, D. W., WILLIAMSON, A. & DE VILLIERS, E. M., 1980. Aetiology of jaagsiekte: Transmission by means of subcellular fractions and evidence for the involvement of a retrovirus. *Onderstepoort Journal of Veterinary Research*, 47, 275–280.

VERWOERD, D. W. & WILLIAMSON, A., 1982. Preliminary characterization of a newly isolated ovine retrovirus causing jaagsiekte a pulmonary adenomatosis. *In:* YOHN, D. S. & BLAKESLEE, J. S. (eds), Advances in comparative leukaemia research 1981. Elsevier North Holland, Inc.

VERWOERD, D. W., PAYNE, ANNA-LISE, YORK, D. F. & MYER, M. S., 1983. Isolation and prelimiary characterization of the jaagsiekte retrovirus (JSRV). *Onderstepoort Journal of Veterinary Research*, 50, 309–316.

WEISS, M. J., SWEET, R. W. GULATI, S. C. & HARTER, D. H., 1976. Nucleic acid sequence relationships among "slow" viruses of sheep. *Virology*, 71, 395–401.

Printed by and obtainable from the Goverment Printer, Private Bag X85, Pretoria, 0001