A Paralytic Disease in Nude Mice Associated with Polyoma Virus Infection

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SUMMARY

Nude mice (nu/nu), heterotransplanted with human tumours and kept in isolators, were found to suffer from wasting and posterior paralysis. Electron microscopy of spinal cord tissue revealed virus particles in the oligodendrocytes consistent in size (35 to 40 nm), morphology and distribution with those of the polyoma-SV40 sub-group of papovaviruses. Serology and restriction enzyme analysis of the virus genome showed that the virus was the murine polyoma A2 strain. Inoculation of uninfected nude mice with 10⁷ TCID₅₀ of polyoma A2 strain virus produced a similar disease in these mice with wasting and, after 10 to 23 weeks, paralysis of the hind legs of all surviving mice. Extensive myelin disruption was seen throughout the brain stem and sacral region of the spinal cord and high titres of polyoma virus were found in the whole brain (10⁸-⁹ TCID₅₀/brain) and in the spinal cord (10⁶-⁸ TCID₅₀/spinal cord).

INTRODUCTION

The papovaviruses are a group of small, host-specific DNA viruses, and include polyoma and K viruses in mice, SV40 in monkeys and BK and JC viruses in humans. These viruses can persist for long periods, perhaps for life, in tissues of their hosts, and this has been shown for polyoma in mice (McCance, 1981; Rowe et al., 1960) and BK (Heritage et al., 1981) and JC virus in humans (P. M. Chesters, J. Heritage & D. J. McCance, unpublished observations). Although most members of this group are oncogenic in unnatural hosts, only one papovavirus has been associated with disease in its natural host. This papovavirus, JC virus (JCV), causes a rare demyelinating disease, progressive multifocal leukoencephalopathy (PML) (Padgett et al., 1971) in patients with immune deficiencies due either to disease or immunosuppressive therapy (ZuRhein & Varakis, 1974; Gardner, 1977).

After the transplantation of human tumours into nude (nu/nu) mice, the animals were found to suffer from wasting and hind leg paralysis (Sebesteny et al., 1980). The condition was not unlike PML in man (ZuRhein, 1969) and in monkeys (Holmberg et al., 1977). The husbandry of the mice, epidemiological, pathological and electron microscope findings have been described elsewhere (Sebesteny et al., 1980). This paper describes experiments to identify the aetiological agent of the disease in mice, to reproduce the disease in uninfected nu/nu mice with this agent and to compare the pathology with that of PML in humans.

METHODS

Animals. The male nude mice used were from a genetically mixed background. They were kept in a modified isolator with a filtered unidirectional air flow (Sebesteny & Lee, 1973). Food, and sawdust bedding were sterilized by 5 Mrad γ-irradiation, double-wrapped and sealed in plastic bags; drinking water was autoclaved in 500 ml bottles.

Virus. The virus used was polyoma A2 strain (Fried et al., 1975). When the nude mice were 2 to 3 months old they were inoculated with approximately 10⁷ TCID₅₀ (50% tissue culture infectious doses) by the intracerebral route (i.e.) after mild anaesthesia with ether.
Haemagglutination (HA) and haemagglutination inhibition test (HAI). Blood was collected by terminal cardiac puncture and antibodies were measured by HAI as described previously (McCance & Mims, 1977).

Virus isolation in tissue culture. African green monkey kidney (AGMK), human foetal lung fibroblasts (FL), mouse embryo fibroblasts (MEF), baby mouse kidney (BMK) and hamster (BHK/C13) cells were used to isolate the virus. A spinal cord homogenate from a spontaneously affected nude mouse was injected i.c. into nude mice and 7 to 10 weeks later, cutaneous tumours appeared. Two of these were macerated in 5 ml phosphate buffer (without Ca\(^{2+}\) or Mg\(^{2+}\)) under sterile conditions. The resulting suspension was divided between two dishes each of subconfluent monolayers of AGMK, FL, MEF, BMK and BHK/C13 cells using 0.5 ml per 50 mm dish. Control dishes were treated with phosphate buffer only. The cells were incubated for 90 min at 37 °C, then 5 ml of Dulbecco's modified E4 medium containing 5% foetal calf serum was added to all the dishes and they were incubated at 37 °C in a 5% CO\(_2\) atmosphere. After one week, medium was removed from the AGMK, FL and C13 cells (one dish from each), 5 ml of fresh medium was added to the cells and they were kept for a further week at 37 °C. Most of the infected MEF and BMK cells showed a marked cytopathic effect (c.p.e.) after 1 week. They were then frozen and thawed three times, and the virus was assayed by the HA method (McCance & Mims, 1977).

Virus assay. Infectivity titres were assayed in various tissues after they had been homogenized in 1 ml of serum-free culture medium as described previously (McCance & Mims, 1977). Titres were calculated by the method of Reed & Muench (1938) and expressed as TCID\(_{50}\).

Preparation and analysis of DNA. Virus stocks derived from MEF and BMK cells were used to infect 3T6 mouse cells, and virus DNA was prepared as described previously (Griffin et al., 1974). Supercoiled (Form 1) DNAs isolated in each case from a single CsCl density gradient were cleaved with restriction endonucleases HpaII, HinfI and SstI and separated on 1.4% agarose slab gels in 40 mM-Tris–HCl pH 7.5, 5 mM-NaOAc, 1 mM-EDTA buffer, with addition of ethidium bromide. The bands were photographed under ultraviolet light.

Electron microscopy. Animals were killed by ether, perfused with 3% paraformaldehyde and tissues taken and cut into 1 mm cubes. These were fixed for 2 h in 2.5% glutaraldehyde in cacodylate buffer at pH 7.3, washed three times in cacodylate buffer, re-fixed in osmium tetroxide, embedded in epoxy resin (Araldite) and sectioned for electron microscopy. Sections were counterstained with lead citrate followed by uranyl acetate.

Immunofluorescent staining. Pieces of tissue were either frozen in liquid nitrogen and cryostat-sectioned or fixed in formalin, embedded in wax and sectioned. The latter sections were mounted on slides, dewaxed by sequential immersion in xylene and ethanol and dried at room temperature. They were immersed in three changes of phosphate buffer (without Ca\(^{2+}\) or Mg\(^{2+}\)) with 1% Nonidet P40 (Sigma) for 20, 5 and 5 minutes respectively. Direct immunofluorescence was carried out on cryostat sections using fluorescein isothiocyanate (FITC)-conjugated mouse anti-polyoma serum with a titre of 12800 HAI units, prepared as described previously (McCance & Mims, 1977).

Immunoperoxidase staining. Cryostat sections were washed in buffered saline (0.05 M-Tris-buffered saline pH 7.6), incubated at room temperature with a 1/5 dilution of normal swine serum for 15 min, washed in buffered saline and incubated overnight at 4 °C with a 1/100 dilution of rabbit anti-polyoma serum (HAI titre = 6400). After washing in buffered saline, the sections were incubated at room temperature with peroxidase-conjugated swine anti-rabbit serum for 30 min, washed and the reaction was developed with freshly-made 0.03% diaminobenzidine and 0.01% hydrogen peroxide in 0.05 M-Tris-buffered saline. The sections were washed in tap water, counterstained with haematoxylin, dehydrated and mounted in Depex.

Haematoxylin and eosin staining. The whole spinal columns of three mice were cut into 3 mm lengths, fixed in formalin, decalcified, embedded in wax, sectioned, dewaxed and stained with haematoxylin and eosin.

RESULTS

The nude mice affected by the wasting and paralytic disease were at the time being used as hosts for human tumours implanted subcutaneously. A total of 24 mice were affected. Symptoms, incidence and spread in the mice has been presented elsewhere (Sebesteny et al., 1980). Briefly, the mice progressively lost weight, became sluggish and paralysis, initially spastic but later flaccid paralysis, of the hind limbs and tail developed. Four of seven adult nude mice injected i.c. with homogenized spinal cord from a paralysed nude mouse developed subcutaneous tumours and two of these also showed paralysis.

Electron microscopy of brain and spinal cord showed oligodendrocytes with their nuclei containing virus particles (Fig. 1). The virus was of the size and morphology of the papovavirus group.

Serology

Haemagglutination-inhibiting antibodies were tested for in 18 adult euthymic mice from various inbred and outbred strains in the specific pathogen free (SPF) colony of the Imperial
Cancer Research Fund Animal Unit (Mill Hill, London) and also in 24 such mice issued from there and held in the experimental unit (Lincoln’s Inn Fields, London) in three rooms in open cages for 6 to 8 weeks. All were negative (< 1/20) for polyoma antibodies.

Sera were available from 2 of the 24 nude mice affected with the disease and from unaffected mice, both nude and euthymic, that had been kept in the same isolators. The polyoma antibody titre in the two affected nude mice was < 1/20 and 1/20, while the titres in five unaffected mice ranged between 1/20 and 1/80 and in two unaffected mice (nu/+ ) were < 1/20 and 1/640. Sera from four euthymic mice inoculated with homogenized spinal cord from a paralysed nude mouse produced HAI titres of 160 to 320 one to five weeks post-inoculation. Anti-polyoma antibody titres in four adult mice infected with the A2 strain by i.c. inoculation were 1/20, 1/80, 1/160 and 1/320 HAI units 15 to 23 weeks after infection. Those titres that were < 1/20 when tested by HAI were always negative by indirect immunofluorescence even when tested undiluted. Antibodies to polyoma infection produced in nude mice were shown by indirect immunofluorescence to be of the IgM and IgG classes. HAI antibodies against K virus, another papovavirus of mice, were not detected in any affected or unaffected mice tested.

Direct immunofluorescence of subcutaneous tumours

Sections were cut from cutaneous tumours developing in a nude mouse injected with homogenized spinal cord from a paralysed nude mouse. Immunofluorescence observations showed polyoma antigens in the nuclei of cells of the tumours (Fig. 2a). The antiserum used was a hyperimmune serum with antibodies against the VP or structural antigens of polyoma. This suggested that infectious virus particles are present in the nuclei and these were seen by electron microscopy (Fig. 2b).

Isolation of virus in tissue culture

The virus isolated from subcutaneous tumours grew in MEF, BMK and BHK/C13, though slightly less well in hamster than in mouse cells, when measured by the yield of haemagglutinin: the titres were 4096, 8192 and 2048 respectively. Mouse cells are permissive for the growth of
polyoma virus whereas hamster cells are semi-permissive (Topp et al., 1980). No c.p.e. was observed in FL or AGMK cells and no haemagglutinin detected by haemagglutination of guinea-pig RBCs. These characteristics are similar to those of murine polyoma virus and differ from those of SV40 or the human papovaviruses (JC and BK).

**Restriction analysis of DNA of the virus**

Analysis of DNA derived from 3T6 cells infected with virus from nude mouse tissues showed it to be identical (Fig. 3) with DNA obtained from the A2 strain of polyoma virus following analysis with three different restriction enzymes HpaII, HinfI and SstI (Griffin et al., 1974). Virus stocks derived from the nude mouse isolate were of high titre (see above) and devoid of any defective DNA (Fried et al., 1975). Data (not shown) obtained from cleavage of the relevant DNAs with HaeIII and PvuII also indicated that the nude mouse virus was A2 polyoma.
Paralytic disease, polyoma infection and nude mice

Fig. 4. Sections of brain tissue stained by direct immunofluorescence: (a) cerebrum showing fluorescent cells in the medulla of the cerebral hemispheres; (b) cerebellum showing fluorescent cells in the granular layer. Brain tissue stained by the immunoperoxidase method: (c) cerebrum showing a positively-stained endothelial cell (single arrow), a positively-stained brain cell (double arrow) and brain cells with pyknotic nuclei can also be seen (long arrow); (d) cerebellum showing a positively-stained cell in the molecular layer (arrow). Bar marker represents 32 μm for (a) to (d).

Production of paralytic disease in nude mice with polyoma virus

Sixteen 2 to 3 month old nude mice housed in the Department of Microbiology, Guy's Hospital Medical School, were inoculated i.c. with 10⁷ TCID₅₀ of A2 strain polyoma virus. One mouse died 1 week after infection and another at 6 weeks, but autolysis was too far advanced for examination of tissues. Six mice were harvested with extensive tumours before any signs of paralysis were evident. Development of a variety of tumour types in nude mice following polyoma infection is a common occurrence (Allison et al., 1974). The remaining eight mice all began to exhibit wasting by 8 weeks post-infection and developed paralysis between 10 and 23 weeks post-infection. Mice were killed and tissues taken and processed for electron microscopy (brain stem, sacral region of the spinal cord and cerebrum), immunofluorescence and immunoperoxidase staining (cerebrum and cerebellum were fast-frozen in liquid nitrogen), haematoxylin and eosin staining (spinal columns) and the whole brains and spinal cords from four mice for assay of virus titres.
Fig. 5. Electron micrographs of the brain stem. (a) Scattered vacuolation of myelin sheaths (v) amongst otherwise normal appearing tissue. Some Wallerian degeneration can also be seen (w). Bar marker represents 5 μm. (b) Vacuolation of both inner (I) and outer (O) lamellae of myelin sheaths with resulting compression of the axons. Bar marker represents 2.5 μm. (c) Gross disorder of paranodal myelin with detachment of paranodal loop. Bar marker represents 2.5 μm. (d) Disruption of myelin sheath in a nude mouse infected with polyoma but before showing signs of paralysis. Bar marker represents 1 μm.

Direct-immunofluorescence staining of cerebrum and cerebellum showed virus infection in various areas (Fig. 4a, b). Infected cells could be seen in the cerebral hemisphere (Fig. 4a) and in the granular layer of the cerebellum (Fig. 4b). Confirmation that polyoma replicated in various cells in different areas of the brain was observed with immunoperoxidase staining, where infected cells were seen in the endothelial and brain cells of the cerebrum (Fig. 4c) and in the molecular area of the cerebellum (Fig. 4d). Many cells with pyknotic nuclei were seen in the cerebrum and there did appear to be a reduction in the number of oligodendrocytes, although this was not quantified. It was difficult to determine precisely which cells were infected in the
Fig. 6. Haematoxylin and eosin stained transverse section in the lumbar region of the spinal column of a nude mouse exhibiting hind leg paralysis showing (a) a tumour (lower right) growing out from a vertebra and impinging on the spinal cord (upper left) and (b) pyknotic nuclei (arrows) in cells of the spinal cord. Bar markers represent 25 μm in (a) and 10 μm in (b).

brain substance but it has been shown by electron microscopy that polyoma infects oligodendrocytes (Fig. 1). Virus antigens were also detected by both staining techniques in the ependymal layer of the ventricles and in the cells of the meninges.

Toluidine blue-stained sections from resin blocks did not show any plaques of demyelination but when the brain stem and spinal cord were observed by electron microscopy, disruption of the
Table 1. *Titres* of polyoma virus in tissues of infected nude mice exhibiting wasting and hind leg paralysis

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Brain</th>
<th>Spinal cord</th>
<th>Kidney</th>
<th>Urine</th>
<th>Salivary gland</th>
<th>Liver</th>
<th>Spleen</th>
<th>Lungs</th>
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<td>10^8.5</td>
<td>10^8.5</td>
<td>ND</td>
<td>&gt;10^8</td>
<td>10^8.25</td>
<td>&gt;10^8</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
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<td>4</td>
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* Titres expressed as TCID$_{50}$ per organ.
† In these two mice, polyoma DNA was found in spinal cord tissue by DNA–DNA hybridization on nitrocellulose filters.
‡ ND, Not determined.

myelin sheaths was widespread (Fig. 5). Disorder of the myelin sheath resulted in a microspongiform degeneration with distention of the inner oligodendrocyte tongues and consequent compression and distortion of axons (Fig. 5 a, b). Disruption of the myelin sheaths was very pronounced at paranodal regions with a lifting of the paranodal loop (Fig. 5 c). Some Wallerian degeneration was evident in brain stem and sacral region of the spinal cord (Fig. 5 a). At high magnification, in one nude mouse killed before paralysis appeared, ballooning and degeneration of the myelin was observed (Fig. 5 d). Again, by electron microscopy, many pyknotic cell nuclei which have the appearance of oligodendrocytes were observed both in the cerebrum and brain stem. In a few areas microglial cells were evident with large lipid granules in their cytoplasm although the number observed was small compared with the amount of myelin degeneration. No other inflammatory cells were seen in areas of demyelination, nor was there vascular cuffing around nearby blood vessels.

Although no gross tumours in the central nervous system were evident, sequential sections of the spinal column were processed with haematoxylin and eosin staining. Small bone tumours were seen in two out of three spinal columns investigated and only one of these exhibited hind leg paralysis at the time of harvesting. The other mouse was harvested due to extensive tumour formation in other tissues. The paralysed mouse had a bone tumour of the vertebrae which had grown into the spinal canal and impinged on the spinal cord (Fig. 6 a). Also observed in the spinal cord of this mouse were several pyknotic cell nuclei both distal and proximal to the tumour (Fig. 6 b).

Titres of polyoma virus in various tissues were measured (Table 1) with levels of between 10^7.5 and 10^8.5 TCID$_{50}$ per brain. No other virus particles were observed except for occasional endogenous C-type particles. Apart from one mouse mentioned above, tissues from the mice harvested because of the presence of extensive tumours were not processed for electron microscopy because the tumours prevented efficient perfusion and in some cases were present in the central nervous system. Titres of virus in the brain and spinal cord were, however, comparable to those from mice exhibiting hind leg paralysis.

**DISCUSSION**

The human papovavirus JCV infects a majority (60 to 70%) of the population in many countries around the world (Gardner, 1977). No obvious symptoms are evident during acute infection and the virus probably persists throughout life as evidenced by detection in normal human kidneys (P. M. Chesters, J. Heritage & D. J. McCance, unpublished results), and reactivation in women during pregnancy (Coleman *et al.*, 1980) and in immunosuppressed patients (Gardner, 1977). In a very small number of cases this virus causes the demyelinating disease progressive multifocal leukoencephalopathy (PML). The disease has a fatal outcome a few months or years after symptoms appear, and JCV can be seen by electron microscopy in oligodendrocytes (Gardner, 1977). The disease is usually found in persons who have impaired cell-mediated immunity as a result of some concurrent chronic disease or immunosuppressive therapy (ZuRhein, 1969). A similar disease has also been observed in monkeys in association with their papovavirus, SV40 (Holmberg *et al.*, 1977).
This paper reports the finding that polyoma virus can cause a paralytic disease in homozygous nude mice which have an impaired cell-mediated immune response. The disease was first seen in nude mice used for subcutaneous transplantation of pieces of human tumour. Virus particles seen in oligodendrocytes had the size and morphology of the papovavirus group. Subsequent proof that the disease was due to polyoma came from the following results. (i) Serological studies showed that affected and unaffected nude mice and euthymic (nu/+) mice from the same isolators had antibodies to polyoma virus. Antibody levels were low in nude mice perhaps due to the lack of T-cell helper activity (Burns et al., 1975) but were shown by immunofluorescence to be of both IgM and IgG classes. No antibodies to the other papovavirus, K virus, were detected. The stock of mice from which these experimental mice originated were negative for polyoma antibodies. (ii) Tumours appearing in the skin of some paralysed mice (not bearing the human transplanted tumours) contained polyoma virion antigens. (iii) When infectious polyoma virus was isolated from these tumours and grown on mouse cells in vitro and its DNA was analysed by restriction endonucleases, it had the same pattern as A2 strain polyoma virus (Griffin et al., 1974). (iv) Paralysis appeared in two out of seven nude mice inoculated with a spinal cord homogenate from an original affected nude mouse and polyoma virus-like particles were seen in the oligodendrocytes (Sebesteny et al., 1980).

To complete Koch's postulates, a pure stock of A2 strain virus was inoculated by the i.c. route into uninfected nude mice and the disease was reproduced in 8 of 16 animals inoculated. The other animals either died soon after inoculation or were harvested because of extensive tumour production. Paralysis was observed, starting at 10 weeks after infection.

The cause of the paralysis may be due to two effects of polyoma infection in nude mice. Electron microscopy of the brains of the paralysed mice revealed areas of disruption and degeneration of the myelin sheaths, due perhaps to polyoma replication in oligodendrocytes (Fig. 1), although plaques of demyelination were not observed as in PML in humans (ZuRhein, 1969) and monkeys (Holmberg et al., 1977). It may be that plaques would have developed if the mice had been left longer, but all were harvested at the first sign of paralysis. In addition, although the degeneration was extensive throughout the brain and spinal cord, very few lipid-filled microglia were observed; this may mean that the degenerating myelin might remain associated with the axons for long periods. This absence of actively phagocytosing microglia may indicate that damage has only just started or may be because the nude mouse is heavily immunodepressed. The disruption of the myelin sheaths as seen by electron microscopy especially in the paranodal areas may be sufficient in itself to cause the gross clinical signs of paralysis. No inflammatory cells were seen in or around this area of degenerating myelin, which is also the case at the periphery of plaques in PML in humans.

Paralysis in some mice may be caused by the compression of the spinal cord by bone tumours in the spinal column. Two spinal columns, one from a paralysed mouse and one from a non-paralysed animal exhibited small tumours of the vertebrae. In the case of the paralysed mouse, the tumour had grown into the spinal canal (Fig. 6a) and was in contact with the spinal cord. In each mouse only one tumour was detected in sequential sections from the cervical to the sacral region. No tumours were detected in one other spinal column from another paralysed mouse. The tumours, when present, may cause paralysis by pressing on the spinal cord and may be responsible for the Wallerian degeneration seen further up the central nervous system in the brain stem. The presence of tumours in the spinal column, however, can not account for degenerative changes in the myelin around intact axons (Fig. 5) and this pathology may be due to replication of polyoma in oligodendrocytes.

Virus titres in the brain and spinal cord of paralysed mice were high (Table 1) and infected cells of many types, including meningeal, ependymal and endothelial, were found throughout the brain. Virus particles were observed by electron microscopy in oligodendrocytes but not in astrocytes (Sebesteny et al., 1980). There did appear to be a reduction in oligodendrocytes, perhaps due to cell death, during polyoma infection, although a recent study (Kerns & Frank, 1981) has shown that normal healthy homozygous nude mice have up to 28% fewer oligodendrocytes than do heterozygous mice in their spinal cords. In vitro studies (D. J. McCance, unpublished observations) showed that both mouse oligodendrocytes and astrocytes are
productively infected by polyoma virus, as are human astrocytes by JCV, although in situ hybridization on human PML brain tissue (Dörries et al., 1979) indicated that astrocytes contain JCV DNA but no virus particles, indicating an abortive infection. A greater variety of brain cells are infected in nude mice with polyoma virus than in PML in humans, where oligodendrocytes seem to be the only cells that are productively infected.

The A2 strain of polyoma virus infecting the original experimental mice was being used to inoculate rats in an adjacent room and transmission to the nude mice in the isolators is thought to have been through sawdust bedding taken inadvertently from open bins in the rat room to fill up bags which were autoclaved and then used for mice. These bags were double-wrapped in autoclave plastic bags (Sterilin), sealed airtight and autoclaved at 134 °C for 5 min. It is now understood that the manufacturer does not guarantee adequate steam penetration into the contents of the bags if they are not left open during autoclaving. Polyoma is quite resistant to heat (Brodsky et al., 1959) and might have survived such treatment. Also, the fact that no experimental euthymic mice (nu/+) issued from the breeding unit and used in three other rooms adjacent to the rat room contained antibodies to polyoma, even though no particular barrier procedures were used other than routine animal house hygiene, suggested carriage of the virus by some mechanical means such as the sawdust. The mice in these three rooms did not receive any of the sawdust from the rat room. It is thought that the main route of transmission in animal house colonies is via urine-contaminated sawdust (Rowe et al., 1961).

In the animal unit at Guy’s Hospital Medical School, CD-1 outbred Swiss mice have been infected by both intraperitoneal and intracerebral routes with polyoma virus. Over a period of 4 years a small fraction (< 1%) have exhibited hind leg paralysis similar to that seen in nude mice. These mice have been shown to be persistently infected with polyoma, as small amounts of the virus were detected in kidneys (McCance, 1981). Electron microscopy showed myelin degeneration but no polyoma virus could be detected in 12 of these mice by electron microscopy or in explants of brain and spinal cord but polyoma DNA was detected in 4/4 mice by DNA–DNA hybridization techniques (D. J. McCance, unpublished observations). The DNA was present in catenated form and, when digested with EcoRI (which cuts polyoma once), the DNA ran as form III, the linear component of polyoma virus DNA. No tumours or other viruses were found in these animals and it may be that the paralysis in the CD-1 mice is due to polyoma virus in a non-infectious form.

The results show that the mouse papovavirus polyoma can produce a paralytic disease in nude mice. The infection may result in paralysis because of destruction of myelin supporting cells such as oligodendrocytes or by production of vertebral tumours which press on the spinal cord. In any event, the disease has a pathology different from that of PML in humans and monkeys, perhaps reflecting the evolutionary divide between the primate and rodent hosts and their respective papovaviruses.

REFERENCES


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