Persistence and Expression of Marek’s Disease Virus DNA in Tumour Cells and Peripheral Nerves Studied by in situ Hybridization

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SUMMARY

We have used cloned fragments of Marek’s disease virus (MDV) DNA and in situ hybridization to search for virus DNA and study its expression in infected chick embryo fibroblasts (CEF), lymphoblastoid cell lines, tumours and neural lesions. DNA from the HPRS 16/att strain of MDV was cleaved with EcoRI endonuclease and several fragments were cloned in Escherichia coli using the vector PBR322. Seven fragments ranging in size from 2.6 to 11 kbp representing approx. 25 % of the MDV genome were labelled in vitro and annealed to EcoRI digests of DNA from infected cells and tumours following separation and transfer according to the Southern blotting procedure. Most of the selected MDV DNA fragments hybridized to fragments of corresponding sizes in EcoRI digests of DNA from cell lines and tumours and failed to hybridize to digests of uninfected chick cell DNA. In situ hybridization using 3H-labelled DNA with specific activity of 108 d/min/µg as probe showed intranuclear MDV DNA in infected CEF, in every cell of two lymphoblastoid cell lines and in the majority of infiltrating or proliferating lymphoid cells found in type ‘A’ lesions of grossly enlarged peripheral nerves. Both intranuclear and cytoplasmic RNA were detected in cells that contained virus DNA. However, comparatively little virus RNA appears to be transcribed in cell lines and in infected tissues from the regions of virus DNA (25 % of genome) used as probe in this study. Our results favour the hypothesis that the accumulation of lymphoid cells in nerves is not the result of an inflammatory response to infected nerve cells but is rather the consequence of proliferating transformed cells.

INTRODUCTION

Marek’s disease is a lymphoproliferative disease of the chicken caused by a herpes virus (Churchill & Biggs, 1967). Studies on the pathogenesis of the disease (Payne et al., 1976) have shown that soon after infection, Marek’s disease virus (MDV) replicates in the lymphoid organs resulting in the formation of intranuclear inclusions and virus antigens in the thymus, bursa and spleen. Birds recovering from this acute phase develop tumours in visceral organs and commonly a proportion of the chickens develop neurological lesions characterized by lymphoid infiltration – principally in peripheral nerves.

It has been known for some time that MDV persists in tumours, since infectious virus can be rescued by co-cultivation of tumour cells with permissive cells (Churchill, 1968) and...
tumour DNA hybridizes with virus RNA in solution (Nazerian et al., 1973). However, none of these studies has identified the cell type or determined the proportion of cells that carry MDV, and attempts to detect virus antigens or particles in situ have been unsuccessful (Payne et al., 1976). Furthermore, the persistence of virus in nerves has been inferred only from the observation of virus antigens and of particles in explanted cultures (J. S. Stevens, personal communication) and the stimulus for lymphoid infiltration has remained elusive (Lawn & Payne, 1979).

We report here on the persistence and expression of virus DNA in infected chick embryo fibroblasts (CEF), lymphoblastoid cell lines and in gross visceral and neural lesions as studied by in situ hybridization. We have used a sensitive method of hybridization (Brahic & Haase, 1978; Haase et al., 1981) and cloned MDV DNA fragments as probe.

**METHODS**

*Viruses and cells.* HPRS 16 (Purchase & Biggs, 1967) and the plaque-purified JM and GA strains of virulent MDV were grown in duck embryo fibroblasts (DEF) as described previously (Ross et al., 1973). HPRS 16/att (Churchill et al., 1969) and plaque-purified attenuated JM were propagated in CEF or in DEF. Lymphoblastoid cell lines MSB-1 (Akiyama et al., 1973), HPRS-1 (Powell et al., 1974), MKT-1 (Silver et al., 1979) and RPL-1 (Nazerian et al., 1977) were cultured in RPMI medium containing 10% foetal calf serum, 10% tryptose phosphate broth in roller bottles at 38 to 40 °C as described previously (Ross et al., 1977). All the cell lines except RPL-1 contained small numbers of cells which scored as infectious centres when plated on chicken kidney cells: MSB-1 (25 per 10⁹), HPRS-1 (5 per 10⁶) and MKT-1 (< 1 per 10⁶).

*Virus purification.* Infected CEF or DEF showing confluent c.p.e. were harvested by trypsinization, resuspended (10⁶/ml) in 1 mM-phosphate buffer pH 7.4 and left to stand at 0 °C for 30 min. The non-ionic detergent Nonidet P40 (NP40) was added to a final concentration of 1% (v/v). The mixture was homogenized in a Dounce homogenizer until release of nuclei could be observed microscopically. Sucrose was added to a final concentration of 0.25 M and the mixture centrifuged at 10000 rev/min for 10 min in an SW25 rotor (MSE). The supernatant was collected. The pellet was resuspended in phosphate buffer, homogenized and centrifuged as before. Supernatants were pooled and 5 to 7 ml (equivalent to 5 × 10⁸ cells) were layered on a discontinuous sucrose gradient consisting of 5 ml 60% (w/w), 6 ml 20% (w/w) sucrose in TE buffer (20 mM-tris pH 7.6, 2 mM-EDTA) and centrifuged at 20000 rev/min for 1 h in an SW25 rotor. The lower interface was collected, diluted twofold in TE buffer and sonicated to dissociate membranes. Further elimination of cell membranes was achieved by a second centrifugation in sucrose as above. The lower interface was collected, diluted in TE buffer and centrifuged at 25000 rev/min at 4 °C in an SW25 rotor. Virus nucleocapsids banding at a density of 1.311 g/ml were collected, diluted with TE buffer and sedimented at 25000 rev/min for 1 h.

*Extraction of DNA.* Virus DNA was obtained from purified nucleocapsids by treatment with SDS, RNase, Pronase and extraction with phenol and chloroform according to Nazerian et al. (1973). Generally, 20 μg DNA were obtained from 3-0 × 10⁹ infected cells. DNA was obtained from lymphoblastoid cell lines and from a portion of an MDV-induced kidney tumour by standard procedures which involved treatment with SDS, RNase, Pronase, phenol and chloroform (Shank et al., 1978). The yield of DNA from cell lines was usually 3 μg per 10⁶ viable cells.

*Labelling of DNA* in vitro. Purified virus and cloned DNAs served as templates for the synthesis of ³²P-labelled cDNA probe using [α-³²P]dCTP (490 Ci/mmol, New England Nuclear), reverse transcriptase and calf thymus DNA primers (Shank et al., 1978). Probes
for in situ hybridization were synthesized as described above except that \([^{3}H]dTTP\) (100 Ci/mmol, New England Nuclear) was used for labelling. Yields were generally 50 to 60% of input template. Specific activities were \(3.5 \times 10^{8}\) d/min/\(\mu\)g for \(^{32}\)P-labelled probes and \(10^{8}\) d/min for \(^{3}H\)-labelled probes. These specific activities are half the theoretical values and take into account the fact that half of the DNA present in the probe is unlabelled template. \(^{3}H\)-labelled PBR322 was used as a control for non-specific binding of probe in in situ hybridization experiments.

**Restriction enzyme analysis.** Restriction endonuclease \(EcoRI\) was purchased from Bethesda Research Laboratories. Enzyme digestion was carried out as prescribed by the supplier and the extent of digestion monitored by including bacteriophage \(\lambda\) as a marker. Cleavage patterns of viral and tumour DNAs were analysed by electrophoresis in horizontal 0.8% agarose gels. DNA was denatured, transferred to nitrocellulose sheets (Southern, 1975) and hybridized to \(^{32}\)P-labelled probe (2 × 10^6 ct/min) for 2 days at 40 °C as described by Shank et al. (1978). Filters were then washed twice in 0.1% SDS, 0.1 × SSC at 52 °C for 1 h followed by several rinses in 0.1 × SSC (Shank et al., 1978). The filters were subsequently air-dried and exposed to X-ray film (Kodak X-Omat R).

**Cloning of MDV DNA.** All manipulations with recombinant DNA were performed in a P 2 containment facility according to specifications of the NIH Committee on Recombinant DNA. Approx. 300 ng \(EcoRI\)-digested PBR322 DNA were mixed with 2 \(\mu\)g \(EcoRI\)-digested HPRS 16/att DNA and the mixture ligated with \(T_{4}\) DNA ligase (New England Biolabs) in a 20 \(\mu\)l reaction mixture as described by DeLorbe et al. (1980). Half of the mixture was used to transform competent cells of \(E. coli\) strain HB101 (Boyer & Roulland-Dussoix, 1969). Transformed cells were plated on agar containing ampicillin (40 \(\mu\)g/ml) and tetracycline (15 \(\mu\)g/ml). Colonies were screened for MDV inserts by in situ hybridization to replicas on nitrocellulose sheets (Grunstein & Hogness, 1975). Positive colonies were picked and cultured in 500 to 1000 ml vol in the presence of ampicillin and tetracycline. A modified Hirt extraction was performed on the bacteria to obtain partially purified recombinant DNA (Clewell & Helinski, 1969). Superhelical recombinant DNA was further purified by extraction with acid–phenol and chromatography through Biogel A 50 (Bio-Rad) as described previously (DeLorbe et al., 1980). Purified recombinant DNA was digested with \(EcoRI\) for analysis by electrophoresis in agarose gels and for probe synthesis.

**In situ hybridization.** The conditions for detection and quantification of virus DNA and RNA were as previously described for visna virus (Brahic & Haase, 1978; Brahic et al., 1981) and for measles virus (Haase et al., 1981). The addition of dextran sulphate (10%, w/v) to the hybridization mixtures enhanced hybridization and improved sensitivity. Usually, 4 ng of probe were added per slide. Slides were coated with Kodak NTB2 liquid emulsion and exposed at 4 °C for 2 to 14 days.

**Source of infected materials for in situ hybridization.** CEF were infected with cell-associated MDV (JM strain) using 1000 p.f.u./10^6 cells and were harvested by trypsinization after 3 to 4 days at 37 °C when c.p.e was evident. Usually, 20 to 30% of the cells were positive in immunofluorescence tests using convalescent Marek’s disease serum. Cells were deposited on slides using a cytocentrifuge and were fixed in ethanol–acetic acid (3:1) for 15 min at room temperature for RNA detection or in methanol–acetone (1:2) for 4 min at −20 °C for DNA detection. The same procedure was used to prepare cells from MSB-1 and MKT-1 cell lines for in situ hybridization, except that the cells were not trypsinized.

Infected peripheral nerves and gross visceral tumours were obtained from SPAFAS chickens inoculated intraperitoneally with 5000 p.f.u. of the JM strain of MDV. Sciatic and brachial nerves were excised 3 to 4 weeks after infection from birds showing leg or wing paralysis. Both enlarged nerves and nerves of normal size were collected from infected
animals. The nerves were snap-frozen in liquid nitrogen and 10 to 20 μm-thick sections were cut in a cryostat. Sections were mounted on slides and were fixed as described above. Normal nerves obtained from pathogen-free SPAFAS chickens served as controls.

**RESULTS**

**Cloning of MDV DNA**

Digestion of MDV (HPRS 16/att) DNA with EcoRI endonuclease produced approx. 20 fragments (total mol. wt. 96 kbp) ranging from 1.3 to 15 kbp in size (Fig. 1a). A mixture of these fragments was cloned in *E. coli* using the vector PBR322. Thirty % of ampicillin- and tetracycline-resistant colonies contained MDV inserts as shown by hybridization with 32P-labelled MDV DNA. Virus DNA used as probe for screening for MDV-positive colonies was prepared from purified virions produced in DEF and was further purified by isopycnic centrifugation in caesium chloride (Nazerian et al., 1973).

Individual colonies were grown in small volumes of broth containing ampicillin and tetracycline. Recombinant DNA was extracted, digested with EcoRI and analysed by gel electrophoresis. At least 12 of the clones obtained contained inserts of different sizes which accounted for approx. 50% of the genome (not shown). However, DNA from only 7 of the clones was used in this study as probe for in situ hybridization. The cloned fragments selected do not cross-hybridize and are representative of approx. 25% of the MDV genome. The molecular sizes of the cloned MDV fragments were 2.6, 3.0, 3.2, 3.5, 5.1, 5.8 and 11 kbp respectively. None of these hybridized to chick cell DNA but reacted with fragments of corresponding sizes in EcoRI digests of HPRS 16/att DNA (Fig. 1b). However, minor differences in the mobility of some of the bands were found in the patterns obtained with digests of different strains of MDV (Fig. 1b, lanes 7, 8 and 9), but not between a virulent strain and its attenuated derivative (Fig. 1b, lanes 8 and 9). Most of the cloned DNA fragments were also represented in digests of DNA obtained from lymphoblastoid cell lines and from a gross lymphoid tumour localized in the kidney (Fig. 1b, lanes 1, 2, 3 and 4). Of the pieces found in cell lines, the 11 kbp fragment was of special interest in view of its large size and of its contribution to the sensitivity of the in situ hybridization. Some minor differences were noted in digests of DNA from different cell lines, and it is noteworthy that all cell lines including HPRS-1 contained extra fragments not present in virus DNA (HPRS 16/att) from which the clones were derived (Fig. 1b). The significance of these differences is not clear at present and is being investigated.

The number of copies of virus DNA per cell was estimated for the MSB cell line by hybridization to nitrocellulose blots using the 11 kbp fragment as probe and dilutions of unlabelled 11 kbp as standard (Fig. 2). It was calculated that the average number of copies in each cell was approx. 15 assuming that the 11 kbp fragment is not repeated within the virus genome. This estimate is lower than previous reports of 60 copies of virus DNA per cell (Nazerian & Lee, 1974). However, the MSB-1 cell line used in this study has been in culture for several years and may have lost virus DNA in this interval. Reduction in virus expression and of the capacity to rescue infectious virus from cell lines during subculture has been documented (Ross et al., 1977) and is consistent with our present findings.

**In situ hybridization to cultured cells**

The probe used throughout the experiments was a mixture of the seven cloned fragments. In MDV-infected CEF which were used as positive controls for in situ hybridization, virus DNA was localized exclusively in the nucleus (Fig. 3), occasionally in a polar distribution probably in cells undergoing mitosis. Four observations attest to the specificity of hybridization to virus DNA: (i) MDV probe did not hybridize to uninfected CEF (Fig. 3) or
Localization of MDV DNA in infected tissues

Fig. 1. (a) Hybridization of $^{32}$P-labelled MDV probe to EcoRI digests of HPRS 16/att DNA from which the clones were derived. (b) Hybridization of seven $^{32}$P-labelled recombinant MDV DNA fragments (arrows) to EcoRI digests of DNA from: MSB-1 (5 μg, lane 1), RPL-1 (2 μg, lane 2), MKT-1 (5 μg, lane 3), kidney tumour (5 μg, lane 4), bacteriophage λ (1 μg, lane 5), uninfected chick embryo cells (10 μg, lane 6), HPRS 16/att virions (1 μg, lane 7), JM virions (5 μg, lane 8) and JM/att virions (5 μg, lane 9). DNA digests were analysed by electrophoresis in 0.8% agarose and were transferred to nitrocellulose for hybridization. Molecular sizes are indicated in kbp.

normal spleen cells (Fig. 4b); (ii) the number of grains observed in infected cells was greatly reduced if denaturation was omitted or (iii) if cells were treated with DNase; (iv) labelled PBR322 DNA did not hybridize to infected cells.

Two lymphoblastoid cell lines (MSB-1 and MKT-1) were examined for the presence of virus DNA. Previous investigators (Nazerian et al., 1973; Silver et al., 1979) using hybridization in solution estimated that cell lines contained multiple copies of virus DNA per cell but did not exclude the possibility that virus DNA was produced in only a proportion of the cells. The results of our in situ hybridization experiments (Fig. 3b) clearly show that every cell of the MSB-1 cell line contained virus DNA. Similar results were obtained with the MKT-1 cell line (Fig. 4a). From the results obtained with MSB-1 (Fig. 2 and 3) we estimate that the sensitivity of in situ hybridization is approx. 2 grains per DNA copy per 7 day
Fig. 2. Estimation of the number of copies of virus DNA/cell in MSB-1 cell line. $^{32}$P-labelled EcoRI digests of the 11 kbp MDV DNA–PBR322 recombinant DNA was hybridized to EcoRI digests of unlabelled cloned DNA (a) and MSB-1 DNA (b) after separation by electrophoresis in agarose and transfer to nitrocellulose. (a) Lanes 1 to 5 contain 0.4, 0.2, 0.1, 0.05 and 0.025 ng of the 11 kbp fragment of MDV DNA respectively. These were calculated on the basis that 11/15 of total recombinant DNA added in each lane is 11 kbp MDV DNA. (b) Lanes 1 and 2 contain 1.4 and 7 μg MSB-1 DNA respectively. The number of copies of virus DNA per cell was calculated to be 15 from the observation that 1.4 μg MSB-1 DNA (b, lane 1) contains approx. 0.1 ng MDV 11 kbp DNA (a, lane 3). Mol. wt. used for the calculation were $7.3 \times 10^6$ and $1.7 \times 10^6$ for the 11 kbp MDV DNA and for chick cell DNA respectively.

exposure. On this basis, CEF at different stages of infection (Fig. 3a) contained varying numbers of virus DNA copies per cell ranging from a few to considerably more than 150.

Virus RNA was also detected by in situ hybridization. With few exceptions (Fig. 5) RNA was found both in the nucleus and in the cytoplasm and in greater amounts in infected CEF than in lymphoblastoid cell lines. This is consistent with limited synthesis of virus in these cells. Hybridization was inhibited by RNase and was therefore specific for virus RNA.

In situ hybridization to tissues from infected chickens

Tissues for hybridization were obtained from chickens showing leg weakness and wing-drop, usually 3 to 4 weeks after infection. The birds selected for study were paralysed and had gross visceral and neural lesions affecting the gonads, kidneys, brachial and sciatic nerves. Fig. 6 (a) shows hybridization to virus DNA in an enlarged brachial nerve exhibiting massive lymphoid infiltration characteristic of type ‘A’ lesions (Payne & Biggs, 1967). The majority of the lymphoid cells contained MDV DNA present at approximately the same levels as in lymphoblastoid cell lines judging from the grain density, the exposure time and taking into account that many of the cells have been sectioned and do not contain as much DNA as whole intact cells. Nerve cells could not be seen clearly in this preparation due to the severity of the lymphoid infiltration. In contrast, it was possible to observe Schwann cells and fibroblasts (arrows in Fig. 6 b) in the contralateral brachial nerve which was normal in size and only mildly infiltrated. There was no significant hybridization to nerve cell elements in this section [mean grain/cell 2 ± 2 (S.D.)] and virus DNA was found mainly in lymphoid cells (Fig. 6b). Nerve sections from two different birds were examined. The percentage of lymphoid cells that contained virus DNA and the mean grain/cell observed are summarized in Table 1. Although the number of birds studied is small, the results are nonetheless consistent and show that the majority of lymphoid cells in type ‘A’ lesions contain virus DNA
Fig. 3. *In situ* hybridization showing MDV DNA. (a) Infected CEF 4 days after infection at m.o.i. of 0.001; (b) MSB-1 cell line; (c) uninfected CEF. Autoradiographic exposure was (a) 2 days, (b) 7 days and (c) 14 days. Mean grain/cell in (c) is 2 ± 2 (s.d.). Note that MSB-1 cells are pleomorphic and that every cell contains virus DNA [mean grain/cell 30 ± 13 (s.d.)]. From this result and from our estimate of 15 copies of virus DNA/cell (Fig. 2), the sensitivity of hybridization is calculated to be 2 grains/copy/7 day exposure. In (a) arrows 1 and 2 show polar, intranuclear distribution of virus DNA in dividing cells. Arrow 3 shows a cell containing approx. 150 copies of virus DNA. All bar markers represent 10 μm.

present at approximately the same levels as in cell lines. We have also failed to detect virus DNA in sections of nerves obtained 5 to 7 days after infection at a stage which precedes lymphoid infiltration. There was no hybridization to nerve cells from uninfected controls (Fig. 6 c).
Fig. 4. In situ hybridization showing MDV DNA. (a) MKT-1 cell line containing 15 copies/cell. Autoradiographic exposure is 7 days. Mean grain/cell is 27 ± 8 (S.D.). (b) Normal lymphoid (L) and erythroid (E) cells obtained from the spleen of an uninfected bird. Autoradiographic exposure is 7 days. Mean grain/cell is 1 ± 0.9 (S.D.). All bar markers represent 10 μm.

Ovarian and kidney tumours were also examined and were found to be similar to type 'A' lesions seen in nerves with regard to cellular composition and content of virus DNA/cell (Table 1). Fig. 7 shows that approx. 80% of the cells in a large kidney tumour contain virus DNA. Since only 10 to 30% of the cells present in gross lymphomas express Marek's disease tumour-specific antigen (Witter et al., 1975), it may be inferred from our results and from those of Witter et al. (1975) that approx. 50 to 70% of the lymphoid cells present in tumours are infected with MDV but do not express the tumour antigen.

We have found only marginal amounts of virus RNA in tumours and infected nerves. The reason for the apparent lack of transcription in these tissues is not known and is being investigated. It is possible that only certain regions of virus DNA are transcribed in lymphoid cells and that these sequences are not represented in the probe used for hybridization.

DISCUSSION

The principal objective of these studies was to identify the type and proportion of cells harbouring MDV in the tumours and peripheral nerves of infected chickens. We have shown by in situ hybridization that the majority of leukocytes in the extensive infiltrates in grossly enlarged nerves (type 'A' lesions) contain approximately the same number of copies of virus DNA as found in cell lines and in a gross visceral tumour. These results are consistent with the hypothesis that the initial nerve lesion is neoplastic and that demyelination and paralysis are secondary to infiltration by infected leukocytes. We cannot wholly exclude an alternative explanation, that nerve cells are infected and provide a stimulus to host immunity and to lymphoid infiltration. However, this seems less likely since we did not find virus DNA in nerve cells under conditions that should have enabled us to detect a single copy of DNA with 2 weeks of exposure.

The successful outcome of these experiments was predicated on two methodological
advances: (i) cloning MDV DNA to obtain virus-specific probes and (ii) a sensitive method for detection of MDV DNA in cells by in situ hybridization (Brahic & Haase, 1978; Brahic et al., 1981; Haase et al., 1981). The MDV DNA fragments that we used as probes were clearly virus-specific and failed to hybridize with chick cell DNA (Fig. 1 and 3) and, although representative of only 25% of the genome, were of sufficient complexity to allow the detection of one or two copies of DNA per cell. Virus DNA could be demonstrated in infected CEF within a few hours, and optimally 24 h after exposure to photographic emulsion (Fig. 3).

We have shown that most of the lymphoid cells in the tumours and infiltrates in peripheral nerves carry virus genetic information, and we surmise from these observations, the polar distribution of MDV DNA in some cells, and the fact that every cell in lymphoblastoid cell lines contains MDV DNA, that the virus genome is perpetuated by vertical transmission.
Fig. 6. *In situ* hybridization showing association of MDV DNA with lymphoid cells in nerve sections. (a) Section of infected, enlarged brachial nerve with massive lymphoid infiltration characteristic of type 'A' lesions. Virus DNA is present in the majority of lymphoid cells. Mean grain/cell is 15 ± 7 (S.D.). (b) Section of contra-lateral brachial nerve with mild infiltration. Virus DNA is not found in Schwann cells (S) or fibroblasts (F) but is found in approx. 70% of lymphoid cells. Mean grain/lymphoid cell is 12 ± 6 (S.D.). (c) Section of uninfected nerve. Autoradiographic exposure is 14 days for (a), (b) and (c). All bar markers represent 10 μm.

to daughter cells. Whether or not this transfer of virus genetic information involves integration of the virus genome is not clear (Kaschka-Dierich *et al.*, 1979; Tanaka *et al.*, 1978). Virus DNA has been reported to be associated with small and medium
Localization of MDV DNA in infected tissues

Fig. 7. In situ hybridization showing MDV DNA in a section of a large kidney tumour. The majority of the cells are lymphoid and contain virus DNA. Mean grain/cell is 14 ± 5 (s.d.). Constituent elements of the kidney were not observed in this part of the tumour. Autoradiographic exposure is 14 days. Bar marker represents 5 µm.

Table 1. Proportion of cells containing MDV DNA and mean grain/cell in sections of nerves and tumours

<table>
<thead>
<tr>
<th>Material</th>
<th>% Lymphoid cells containing MDV DNA</th>
<th>Mean grain/cell ± s.d.</th>
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<tr>
<td>MDV-infected bird (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enlarged brachial nerve</td>
<td>78*</td>
<td>15 ± 7*</td>
</tr>
<tr>
<td>Large kidney tumour</td>
<td>70</td>
<td>14 ± 5</td>
</tr>
<tr>
<td>MDV-infected bird (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enlarged sciatic nerve</td>
<td>80</td>
<td>14 ± 8</td>
</tr>
<tr>
<td>Ovarian tumour</td>
<td>60</td>
<td>24 ± 7</td>
</tr>
<tr>
<td>Uninfected bird</td>
<td>0</td>
<td>1 ± 0.9</td>
</tr>
<tr>
<td>Spleen</td>
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* Data from one section in each case; autoradiographic exposure 14 days.

macrochromosomes based on analysis of separated chromosomes by hybridization (Hughes et al., 1980). In this study we showed that all seven cloned MDV fragments were present in the lymphoblastoid cell lines and in a kidney tumour (Fig. 1 b). The additional bands seen in EcoRI digests of MDV DNA in cell lines and the kidney tumour compared to virus DNA extracted from virions (Fig. 1 b, lanes 3, 4 and 7) are certainly consistent with integration of the virus genome and this possibility is under investigation.

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