Deoxyribonucleic Acid of Marek's Disease Virus in a Lymphoblastoid Cell Line from Marek's Disease Tumours

(Accepted 2 August 1974)

SUMMARY

DNA extracted from a chicken lymphoblastoid cell line (MSB-1) originally derived from a Marek's disease tumour was examined for the presence of the Marek's disease virus genome. DNA cRNA membrane filter hybridization experiments established the presence of 60 to 90 genome equivalents in these cells.

Marek's disease (MD) is a lymphoproliferative disease of chickens which is caused by a herpesvirus (Churchill & Biggs, 1967; Nazerian et al. 1968). Lymphoid tumours of MD are usually free from virus specific antigens and virus particles. Virus genome is, however, present in tumour cells (Nazerian et al. 1973) but is not fully expressed. Two chicken lymphoblastoid cell lines from tumours of MD are now established (Akiyama, Kato & Iwa, 1973; Akiyama & Kato, 1974), but virus specific antigens and virus particles are detected in only 1 to 2% of the cell population. The purpose of the present study was to investigate the presence and extent of the virus DNA in these cells.

Marek's disease virus (MDV) strain GA was used for preparation of DNA and MDV complementary RNA (cRNA). This strain was previously shown to infect duck embryo fibroblast (DEF) cultures and cause the release of large number of virus particles (Lee et al. 1973). The virus was propagated in roller bottle cultures of DEF and was labelled with [³H]-thymidine for 24 h. The fluid from infected cultures was harvested 72 h after infection and was centrifuged at 10000 g to remove the cellular debris. Virus particles were then precipitated by the method described by Adams (1973). Briefly, the clarified culture fluid was brought to 8% polyethylene glycol and 0.5 M-NaCl and incubated at 4°C for 1 h, and then centrifuged at 8000 g for 10 min in a GSA Sorval rotor. The pellet was suspended in virus buffer (0.02 M-tris, 0.15 NaCl, pH 7.5) and was twice centrifuged at 79000 g for 45 min on a 12 to 52% (w/w) sucrose gradient in a Spinco SW-27 rotor. The resulting virus band was recovered with a syringe from the side of the tube, diluted out in virus buffer and was pelleted by sedimentation at 86000 g for 45 min in a Spinco 60 TI rotor. The pellet was then suspended in virus buffer and was used for preparation of virus DNA. Fig. 1 is an electron micrograph of the purified virus used for extraction of virus DNA. Many virus particles, either in the form of aggregates or as individuals were seen in this preparation.

[³H]-DNA was extracted from purified virus and was further purified according to the technique described previously (Nazerian et al. 1973). Briefly, two cycles of caesium chloride density gradients in the angular rotor (Spinco 40) were applied in purification of the DNA. Purified virus DNA was used for preparation of complementary RNA (cRNA). The reaction mixture (1.0 ml) consisted of [³H]-MDV DNA, 0.15 M-KCl, 0.04 M-tris-hydrachloride (pH 8.0), 0.01 M-MgCl₂, 5 × 10⁻⁴ M-dithiothreitol, 10⁻⁴ M-EDTA, 6 × 10⁻⁴ M-[³H]-ATP (sp. act. of 22.6 Ci/mmol), 2 × 10⁻⁴ M-GTP, 2 × 10⁻⁴ M-UTP, 5 × 10⁻⁵ M-CTP, 10% glycerol, and 8 μg Escherichia coli RNA polymerase (Miles Laboratories, Elkhart, Indiana, 21
Fig. 1. An electron micrograph of the negatively stained preparation of the highly purified MDV. Many naked nucleocapsids are seen in this micrograph.

U.S.A.). The mixture was incubated at 35 °C for 3 h. cRNA product was treated with DNase and was twice extracted with phenol as previously described (Nazerian et al. 1973). It was then purified by gel filtration on Sephadex G75 and was stored at -20 °C.

Uninfected chicken embryo fibroblast (CEF) cultures and MDV infected DEF cultures were prepared as described elsewhere (Solomon et al. 1968). A chicken lymphoblastoid cell line (MSB 1) established from an MD tumour kindly provided by Dr S. Kato, was propagated in medium RPMI 1640 with 10 % bovine foetal serum in Falcon plastic Petri dishes. Cultures were incubated at 41 °C in 5 % CO₂ humid atmosphere. DNA preparation from uninfected CEF culture, CEF culture highly infected with GA-MDV; and MSB 1 lymphoblastoid cell line were made by standard procedures employing pronase treatment and phenol extraction (Pettersen & Sambrook, 1973).

DNA cRNA hybridization procedures were those of Birnstiel, Sells & Purdom (1972). Ten µg of heat denatured DNA from different sources was fixed to 13 mm membrane filters (Carl Scheicher and Schuell, Inc., Keene, N.H., U.S.A.). Filters were baked at 80 °C for 4 h and then incubated for 72 h at 42 °C with MDV cRNA (approx. 10000 ct/min/filter) in 0.5 ml of 50 % formamide in 6 × SSC. They were then treated with 0.2 ml of 20 µg/ml RNase (Worthington Biochemical Corp., Free, N.J., U.S.A.) for 30 min at 37 °C, washed twice in 6 × SSC and counted in a Beckman LS 100 scintillation counter. For hybridization calibration experiment, known quantities of [³H]-MDV DNA were mixed with 10 µg of calf thymus DNA, heat denatured, fixed to membrane filters and incubated with MDV cRNA as above. Counts for hybridization calibration profile were corrected for residual counts due to [³H]-MDV DNA.

Results of hybridization experiments using DNA from uninfected CEF, CEF cultures productively infected with GA-MDV (37 % of cell population was positive for virus
Table 1. Hybridization of $[^3H]$-MDV cRNA with DNA from MSB 1 chicken lymphoblastoid cell line, MDV infected chicken embryo cultures and uninfected control cultures

<table>
<thead>
<tr>
<th>DNA source</th>
<th>IF antigen (% positive)</th>
<th>$[^3H]$-MDV cRNA bound per 10 µg of DNA (cpm)</th>
<th>MDV genome equivalents per diploid cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninfected CEF</td>
<td>—</td>
<td>147</td>
<td>—</td>
</tr>
<tr>
<td>MDV infected CEF</td>
<td>37</td>
<td>1882</td>
<td>—</td>
</tr>
<tr>
<td>MSB 1 DNA</td>
<td>1</td>
<td>654</td>
<td>90</td>
</tr>
<tr>
<td>MSB 1 DNA</td>
<td>1</td>
<td>479</td>
<td>60</td>
</tr>
</tbody>
</table>

Fig. 2. Hybridization calibration of $[^3H]$-labelled MDV cRNA with MDV DNA. Known quantities of MDV DNA were mixed with 10 µg of calf thymus DNA, heat denatured, and fixed to nitrocellulose filters. Hybridization procedures are given in the text.

The number of genome equivalents in MSB 1 line was calculated from Fig. 2 where the increase in hybridized counts is directly proportional to the concentration of MDV DNA (assumed 60% pure). The values of $1 \times 10^8$ for MDV DNA mol. wt. (Lee et al. 1971) and $1.7 \times 10^{12}$ for chicken cell DNA mol. wt. (Atkin et al. 1965) were used for this calculation. From data given in Table 1, an average of 60 to 90 genome equivalents per MSB 1 cell was estimated. Results of further experiments fell between these two values. These values are clearly higher than the range of 3 to 15 genome equivalents reported for virus induced tumours of MD in chickens (Nazerian et al. 1973). However, the number of MDV genomes in tumour cells was estimated on the assumption that all cells in the tumour carry virus genome. This assumption may not be valid, and the lower number of virus genomes reported
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for MD tumours may be due to the fact that lymphoid tumours of MD are heterogeneous. The amount of virus genome in all tumour cells may not be equal, and furthermore, many cells within the tumour may not contain any virus DNA, and therefore, lower the estimated number of virus genomes per tumour cell. On the other hand, cells in the lymphoblastoid cell line are homogeneous in size and morphology and show similar antigenic characteristics after in vitro cloning (Akiyama & Kato, 1974). The values reported here must, therefore, be closer to the true value than those reported for the tumours. However, cells in the lymphoblastoid line that are actively supporting the replication of virus DNA may slightly raise the estimated number of genomes per cell.

It is interesting to note that induction of the MSB I cell line with 5-IUDR and 5-BUDR (K. Nazerian, unpublished data) causes the expression of virus DNA and production of virus specific antigens in a higher number of cells (up to 15 %) than in normal untreated cells. These induction experiments show that resident virus genome is present in more than 1 % of the population normally allowing the replication of the virus.

The results presented in this study provide further evidence for the role of MDV DNA in in vitro transformation of chicken lymphoid cells. The status of MDV DNA and its association with host cell DNA is not known. Human lymphoblastoid cell lines transformed by Epstein–Barr virus (EBV) are already shown to contain variable copies of the virus genome (Nonoyama & Pagano, 1971; Zur Hausen et al. 1972). Furthermore, in one human lymphoblastoid line, a linear association is shown, between EBV DNA and host cell DNA (Adams, Lindahl & Klein, 1973).

We thank Dr Shiro Kato for MDV transformed MSB I cell line and we also thank Dr John A. Boezi for the use of his facilities.

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REFERENCES


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(Received 28 May 1974)