Morphological Study of Virus-like Particles in Two Transplantable Tumours from BDX Rats

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SUMMARY

Virus-like particles were found in two transplantable tumours, Sp56 and Sp6, from BDX rats. Sp56, a neurogenic sarcoma, contains abundant C-type particles in all stadia of morphogenesis. This tumour reacts with anti-Friend leukaemia virus gp70 and anti-Rauscher leukaemia virus p30 sera. Sp6, a fibrosarcoma, has abundant virus-like particles in the cytoplasm, very often associated with centrioles or basal bodies of a cilium. These particles consist of two concentric shells with a diam. of 60 to 65 nm. Released particles were found outside the cell with a diam. of 85 to 100 nm characterized by an envelope and an eccentrically located electron-dense nucleoid, surrounded by an intermediate layer. These virus-like particles show no cross-reaction with antisera against murine C- or B-type particles, but show ultrastructural similarity with virus particles recently described in Chinese hamster cells and in mouse cell lines infected with two retrovirus isolates from South-East Asian mice.

In this brief report we describe virus-like particles in two transplantable tumours from BDX rats. The BDX rats were found to be a very high (66%) tumour incidence strain; they developed a variety of spontaneous tumours derived from connective tissue, bone, skin, lung, gastrointestinal tract, genitourinary tract, mammary gland, endocrine glands, neural system and lymphoreticular system (Zöller et al., 1978). Eighty-three% of the primary tumours were transplantable.

Several transplantable tumours were kindly given to us by Dr Zöller (German Cancer Research Centre, Heidelberg, F.R.G.) of which we used four: Sp3 (pheochromoblastoma in 11th generation), Sp6 (fibrosarcoma in 18th generation), Sp50 (neurogenic sarcoma in 37th generation) and Sp56 (neurogenic sarcoma in 49th generation).

Small pieces of the tumours were fixed in 2% glutaraldehyde, post-fixed in 1% osmium tetroxide, dehydrated and embedded in a mixture of epon and araldite. Thin sections were stained with uranyl acetate and lead hydroxide. The preparations were examined with Philips electron microscopes EM-300 and EM-301.

In Sp3 and Sp50 no virus-like particles were found. Tumour Sp56 contained many virus particles similar to C-type particles in all stadia of morphogenesis: budding, immature and mature type (Fig. 1 a to c).

Tumour Sp6 showed many cytoplasmic virus-like particles consisting of two concentric shells, the inner one being more electron-dense than the outer (Fig. 1 d, e). The diam. of the outer shell was 60 to 65 nm, while the diam. of the core was 30 to 35 nm. Very often these particles were found associated with centrioles or the basal bodies of a cilium. Some of these particles were located near the cell membrane (Fig. 1 f). Released retrovirus-like particles were found outside the cell (Fig. 1 g), consisting of an envelope and an eccentrically located electron-dense nucleoid. Between the nucleoid and the envelope an intermediate layer was present. The outer diam. of these particles was 85 to 100 nm.

Cultured lines derived from Sp6, Sp50 and Sp56 were tested in a complement-dependent microcytotoxicity assay for sensitivity to antisera against Friend leukaemia virus gp70, Rauscher leukaemia virus p30, murine mammary tumour virus (MuMTV) whole virion, and MuMTV gp52 (Table 1). Details of the method were described elsewhere (Kuzumaki et al., 1980).
Fig. 1. (a to c). Tumour Sp56. (a) Two budding particles (arrows) and an immature C particle (I). (b) Immature C particle consisting of two concentric shells (1) surrounded by an envelope (2). (c) Mature C particle with an electron-dense nucleoid (1) surrounded by an envelope (2). (d to g) Tumour Sp6. (d) Virus-like particles consisting of two concentric shells (small arrows) near the centriole (C). Microtubules (large arrows) are visible in between the particles. (e) Virus-like particles (arrows) around the basal body of a cilium (B). (f) Virus-like particle (arrow) near the cell membrane. (g) Extracellular particles with a nucleoid (1); in the marked particle two superimposed nucleoids seem to be present, an intermediate layer (2) and an envelope (3).
Table 1. Sensitivity of spontaneous BDX rat tumours to antisera prepared against murine C-type and B-type virus-associated antigens

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Anti-MuLV gp70</th>
<th>Anti-MuLV p30</th>
<th>Anti-MuMTV Whole virion</th>
<th>Anti-MuMTV gp52</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sp3</td>
<td>- - - -</td>
<td>- -</td>
<td>NT†</td>
<td>NT</td>
</tr>
<tr>
<td>Sp6</td>
<td>- - -</td>
<td>- - -</td>
<td>- - - - -</td>
<td>- - - - -</td>
</tr>
<tr>
<td>Sp50</td>
<td>- - -</td>
<td>- - -</td>
<td>- - - - -</td>
<td>- - - - -</td>
</tr>
<tr>
<td>Sp56</td>
<td>40, 10, 80</td>
<td>40, - -</td>
<td>- - - - -</td>
<td>- - - - -</td>
</tr>
<tr>
<td>RBL-5</td>
<td>20480</td>
<td>80</td>
<td>-</td>
<td>NT</td>
</tr>
<tr>
<td>TA3St</td>
<td>5120</td>
<td>640</td>
<td>640</td>
<td>1280</td>
</tr>
<tr>
<td>Balb/3T3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Reciprocal of serum dilution producing more than 0.20 of cytotoxic index. Three repeated experiments.
† No positive reaction at 1:10 serum dilution.
‡ NT, Not tested.

1978). As controls, a Rauscher leukaemia virus-induced murine leukaemia RBL-5, a mouse spontaneous mammary tumour TA3St and a mouse embryonal fibroblast Balb/3T3 were used. In line with the morphological findings, tumour Sp56 reacted with anti-murine leukaemia virus (MuLV) gp70 serum, and once with anti-MuLV p30 serum also. The other three lines, Sp6, Sp3 and Sp50, did not react with the anti-gp70 and anti-p30 sera. None of the lines tested (Sp6, Sp50 and Sp56) were sensitive to anti-MuMTV whole virion and anti-MuMTV gp52 sera. These serological results suggest that the C-type particles from tumour Sp56 share interspecies antigens with other mammalian C-type particles as shown for other rat C-type particles (Sarma et al., 1973). The virus particles produced by tumour Sp6 are antigenically different from murine C-type or B-type viruses.

C-type virus particles have been isolated from a wide variety of mammalian species. In certain species, such as mice and cats, C-type particles are wide-spread and can be found readily in primary, spontaneous or in transplanted leukaemias, lymphomas, sarcomas and fibrosarcomas and in many cases have been proven to be the aetiological agents. In these species, C-type particles are also found in normal tissue and in other types of tumours, for example mammary tumours, probably as a passenger. In certain other species such as rats or dogs, it is usually not possible to find C-type virus particles in primary spontaneous leukaemias, in malignant lymphomas or in other spontaneously occurring tumours.

There are several reports of virus particles observed in rat leukaemias or rat tumours; however, with only a few exceptions, they refer to rat leukaemia or to rat tumours that have been transplanted by cell grafts for a number of generations (for review, see Gross et al., 1975). The possibility cannot be excluded that extraneous virus particles unrelated to the primary tumour have been 'picked up' in the course of successive transplantations from some of the rat hosts and that such particles might have been carried in the grafted tumour cells. At present, we do not know whether the C-type particles found in transplantable Sp56 neurogenic sarcoma and/or the virus particles in transplantable Sp6 fibrosarcoma are responsible for the high incidence of tumours in BDX rats or are simply passengers in these tumours, picked up from the hosts during transplantations. In every case, however, they are rat viruses.

The virus particles found in Sp6 fibrosarcoma have not been described before in rat cells. These particles resemble the particles described in Chinese hamster cell lines, CHO (Wheatley, 1974; Heine et al., 1979) and E36 (Calafat & Hilkens, 1978), and in mouse cell lines infected with two retrovirus isolates from South-East Asian mice (Heine & Todaro,
1978). The similarities are (i) the shape of the intracytoplasmic particles and their association with microtubules and centriole (in the case of the particles of Sp6 also in association with the basal bodies of a cilium) and (ii) the morphology of the released particle with a centrally or eccentrically located nucleoid and an intermediate layer between nucleoid and envelope.

There is some discrepancy about the diameter of the particles. The particles described by Heine in CHO cells and in the two virus isolates from South-East Asian mice have a larger diameter than the particles described in E36 and Sp6. It is possible that these differences in size are real differences between virus particles in different animal species. On the other hand, the differences could be induced by the preparation and measuring techniques, since the same intracytoplasmic particles of the CHO cells described by Wheatley (1974) as having a diam. of 55 to 64 nm are described by Heine et al. (1979) as being 60 to 75 nm in diam.

At present, we have only morphological and immunological data concerning the virus particles found in Sp6 since they were found in quantities too small to perform biochemical investigations. But the ultrastructural similarity with the virus particles recently described in Chinese hamster cells (Heine et al., 1979; Calafat & Hilkens, 1978) and in mouse cell lines infected with two retrovirus isolates from South-East Asian mice (Heine & Todaro, 1978) can justify its classification in the same group. If the presence of a 70S RNA-dependent DNA polymerase could be demonstrated in the virus particles from the hamster cells and from the Sp6 rat tumour, then it would be possible to introduce a new class of Retroviridae.

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REFERENCES

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