OK 10 Virus, an Avian Retrovirus Resembling the Acute Leukaemia Viruses

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SUMMARY

The OK 10 virus complex was isolated from a liver tumour of a chicken which, as an embryo, had been inoculated intravenously with a field isolate of an avian leukosis virus. The OK 10 virus complex contains at least two viruses: the interference assay and serum neutralization test indicate that the helper virus belongs to subgroup A. One of the viruses, OK 10 V, induces distinct foci in chick embryo cells under agar overlay and cells from the foci form colonies in soft agar. These properties allow in vitro assay of the virus. Injection of virus or infected cells into chicks induces acute leukaemia but no local tumours. Another virus, OK 10 AV (associated virus), comprises about 99% of the OK 10 complex. This virus does not induce foci in chick embryo cells. In chickens it causes leukosis 17 months after injection. Electron micrographs of OK 10 virus stocks show typical C type virus particles. These particles have a density of 1.16 g/ml and contain 70S RNA which, after heat denaturation, releases type b RNA subunits. The OK 10 virus complex apparently represents a strain of acute leukaemia viruses.

INTRODUCTION

It has recently been suggested that avian retroviruses can be divided into three main groups (Hanafusa 1977): (i) the sarcoma viruses Rous sarcoma virus (RSV), avian sarcoma virus B77 and the Fujinami viruses, (ii) the lymphoid leukosis viruses, the different resistance-inducing factor viruses (RIF) and Rous associated viruses (RAV) and (iii) the acute leukaemia viruses of which avian myeloblastosis virus (AMV), avian erythroblastosis virus (AEV) and avian myelocytomatosis virus MC 29 are well studied. This corresponds well to earlier classifications by Temin (1971) and Coffin (1976).

The avian sarcoma viruses cause distinct foci in chick embryo cells in vitro. The leukosis viruses have generally been considered non-transforming because none of the established laboratory strains of the different subgroups of helper viruses cause transformation of cultured chick embryo cells (see review by Pontén, 1971). Many of these viruses do, on the other hand, cause a slight cytopathic effect in vitro (Dougherty & Rasmussen, 1964; Graf, 1972; Kawai & Hanafusa, 1972; Oker-Blom et al. 1975). A simple plaque test based on this property has been developed for members of subgroups B and D (Graf, 1972; Kawai & Hanafusa, 1972). After prolonged cultivation of cells infected with many of these viruses, morphological changes in the cultures ("conversion") occur (Calnek, 1964; Oker-Blom et al.)
1975). These changes are different from transformation since converted cells do not exhibit loss of contact inhibition, do not form colonies in soft agar and do not induce solid tumours at the site of inoculation in chickens (Oker-Blom et al. 1975).

Of the viruses classified as acute leukaemia viruses, AMV, originally isolated in 1941, (Hall et al. 1941) induces transformation of haematopoietic cells in culture (Beaudreau et al. 1960; Baluda & Goetz 1961; Moscovici & Vogt 1968; for review see Moscovici 1975). Avian erythroblastosis virus (AEV; Engelbreth-Holm & Rothe-Meyer, 1932) has recently been shown to transform both chicken erythroblasts and fibroblasts in vitro (Graf et al. 1976). The myelocytomatosis virus (MC 29), isolated in 1964 (Ivanov et al. 1964) transforms bone marrow cultures (Todorov & Yakimov 1967; Langlois et al. 1969) as well as chick embryo cells (Langlois et al. 1967). All these viruses contain a transforming virus and an excess of non-transforming helper virus. In the case of AMV, the myeloblastosis associated viruses MAV-1 and MAV-2, belong to subgroups A and B respectively (Moscovici & Vogt, 1968). The AEV helper virus from the two strains studied belongs to subgroup B (Graf et al. 1976). Stocks of MC 29 virus have been found to contain helper viruses of subgroups A and B (Graf, 1972).

Another virus which has recently attracted attention is the MH 2 virus (Begg, 1927). The virus belongs to subgroup C and induces endotheliomas in chickens and foci in chick embryo cells in vitro (Payne & Biggs 1970).

We have isolated (Oker-Blom et al. 1970) a group A avian retrovirus OK 10 which induces rapid transformation and focus formation of chick embryo cells in vitro as well as rapid tumour formation in new-born chicks. The lesions induced in chicks resemble those of the MH 2 virus. Here we describe the isolation and preliminary characterization of OK 10 virus.

**METHODS**

*Chickens.* Brown Leghorn chickens (C/O or C/E cell type) were used. This flock originated from eggs brought to this Institute in 1969 from Houghton Poultry Research Station, Huntingdon, England. As evident from continuous RIF (resistance inducing factor), COFAL (complement fixation avian leukemia) tests and, more recently, RIA (radio-immunoassay) and antibody assays, it has remained leukosis-free (Sandelin & Estola, 1975).

*Cell cultures.* Primary chick embryo cells (CEC) were prepared from 10- to 11-day-old embryos. The growth medium (Medium 199) was supplemented with 10% tryptose phosphate broth, 5% foetal calf serum, penicillin (200 International units/ml) and streptomycin (200 μg/ml). The cells were grown at 37°C in a humidified atmosphere (95% air and 5% CO2) and passaged after 3 to 6 days.

*Viruses.* RAV-1, RAV-2 and RSV (RAV-1) were obtained from Dr H. Hanafusa, New York, N.Y., U.S.A. RSV (RAV-2), RSV (RAV-7) and RSV (RAV-50) were obtained from Dr P. Vogt, University of Southern California, Los Angeles, Calif., U.S.A. OK 10 virus was isolated from an embryo of a leukotic hen (Oker-Blom et al. 1970). The passage history of the virus is described below.

*Interference assays.* Interference assays were performed according to Rubin (1960). RSV (RAV-1), RSV (RAV-2), RSV (RAV-7), RSV (RAV-50) of subgroups A, B, C and D respectively, OK 10 and OK 10 V were used as challenging viruses. OK 10 V was obtained from a continuous cell line derived from a tumour-bearing chicken which seems to produce only OK 10 V (Oker-Blom et al. unpublished data). The standard interfering viruses were RAV-1, RAV-2, RAV-49 and RAV-50 of subgroups A, B, C and D respectively.

*Colony formation in soft agar.* The method used was that described by Macpherson (1969).
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Primary chicken embryo fibroblasts \((10^6)\) were exposed to \(2.5 \times 10^4\) focus forming units (f.f.u.) of OK 10 virus for 2.5 h and colonies counted after incubation for seven days.

**Neutralization tests.** The tests were performed as described previously (Oker-Blom et al. 1975). Control serum was obtained from a leukosis-free Brown Leghorn hen. Anti RAV-1 serum, anti RAV-2 serum, anti RAV-49 serum and anti RAV-50 serum were obtained from Dr. P. M. Biggs, Houghton Poultry Research Station, Huntingdon, England. Anti OK 10 and anti OK 10 AV sera were obtained from chickens infected intravenously at 2 to 4 days of age with 1 ml virus with titres of \(10^4\) f.f.u./ml (OK 10 V) and \(10^6\) interfering units/ml (OK 10 AV). The chickens were bled at 1 month of age.

**Test for Marek's disease virus A-antigen.** The test was performed by Dr. Bodil Norrild, Institute for Medical Microbiology, University of Copenhagen (Norrild & Vestergaard, 1978). No Marek virus A-antigen could be detected.

**Labelling and purification of virus and RNA.** OK 10 virus infected CEC (thirty 10 cm culture dishes) were labelled with 20 mCi \(^{32}\)P-orthophosphate (Institute of Atomenergi, Kjeller, Norway) for 20 h. The supernatants were clarified at 10000 g for 20 min at 4 °C. The virus in the supernatant was centrifuged in an SW 27 rotor at 24000 rev/min for 60 min at 4 °C. The pelleted virus was resuspended overnight in TN (0.05 M-tris, pH 7.4, 0.1 M-NaCl) at 0 °C and 2 ml samples were layered on discontinuous sucrose gradients consisting of: (from the bottom) 0.5 ml 65 % (w/w) sucrose, 2.5 ml + 2.5 ml 30 to 60 % (w/w) sucrose and 4.5 ml + 4.5 ml 10 to 30% (w/w) sucrose in TN.

Purified virus from the discontinuous sucrose gradient band was diluted with TN to a final sucrose concentration of 10% and pelleted in a SW 27 rotor at 24000 rev/min for 60 min at 4 °C. The pellet was taken up in 1 ml of TSE containing 1 % SDS.

The RNA released with SDS was run in an SW 27 rotor on a 15 to 30 % (w/w) sucrose gradient in TSE-buffer at 24000 rev/min for 6 h at 20 °C. The fractions containing RNA were pooled and the RNA precipitated with ethanol at \(-20 °C\) overnight. The precipitate was pelleted and resuspended in 100 μl of 1 % SDS. This RNA preparation was used for gel electrophoresis. A part of the RNA probe was heat denatured for 1 min at 100 °C, and was then cooled in ice. Cylindrical 2% polyacrylamide-0.5% agarose gels supplemented with 0.1% SDS were prepared according to Peacock & Dingman (1968). The gels were run for 3 h at 200 V (10 V/cm), sliced into 2 mm pieces and the radioactivity solubilized with NCS, tissue solubilizer (Amersham/Searle). The radioactivity was determined in a Packard liquid scintillation counter.

**Pathogenicity of OK 10 V and OK 10 AV in chickens.** A group of nine new-born chickens were infected intravenously with different dilutions of RK 10 virus. Three chicks received \(6 \times 10^3\), three \(6 \times 10^5\) and three \(6 \times 10^4\) f.f.u. In another experiment, \(10^6\) interfering units of OK 10 AV virus were injected intraperitoneally into 7 new-born chickens. The chickens were bled at regular intervals, and they were studied for viraemia and neutralizing serum antibodies.

**RESULTS**

**Origin of OK 10 virus**

During a field study in 1969 a leukemia virus was isolated from the embryonated egg of a leukotic hen with lymphoid leukemia. Cell cultures from this embryo were passaged in Roux bottles several times and also studied by electron microscopy. The cells contained C type particles in abundance. In order to save the virus for later experiments the cells from one bottle were frozen. About one year later cells were thawed, homogenized and the supernatant used for infection of leukosis-free chick embryo cells. In order to study the
effect of the virus in vivo some 12-day-embryonated eggs of Brown Leghorn chickens of strain C/O or C/E were injected intravenously with the supernatant fluid from infected cells. The embryos were allowed to hatch and kept in isolation. One of the chickens became ill and was sacrificed as moribund at the age of 17 months. Autopsy revealed lymphoid leukosis and a tumour in the liver. Histologically the liver tumour was a hepatic cell adenoma or carcinoma. Between these cells lymphocytes and myeloid cells were dispersed. A suspension was made from the liver tumour and inoculated on to monolayers of chick embryo cells from the same line of chickens. The virus obtained from such monolayers caused distinct foci in chick embryo cells under agar overlay and is called OK 10.

Clone purified stocks of OK 10 virus

For clone purification of OK 10 virus, 3 ml of the stock liver suspension was inoculated on to monolayers of secondary cultures of CEC of the C/O or C/E line containing about 6 million cells and the cells were passaged. From the sixteenth cell passage the supernatant was used to infect new cells which were overlaid with agar. Ten foci were picked, suspended in 5 ml of growth medium and used to infect new CEC. After five clonings, using one or two foci each time, stock virus was prepared.

Transformation and focus formation by OK 10 virus

In early experiments, stock virus induced regions of morphologically altered cells in monolayers of CEC after only one or two passages (Fig. 1b). This contrasts with the much later conversion of cultures induced by the non-transforming leukosis viruses (Oker-Blom et al. 1975). Under agar overlay the virus induced distinct foci (Fig. 1c and d) which were quite different from foci induced by several RSV strains tested in our laboratory (Fig. 1e). The borders of the OK 10 foci were more irregular than those induced by RSV: the cell looked less distinct but they never seemed to disappear as did the cells of the RSV (RAV-1) foci in which a 'hole' was often formed. With experience it was easy to distinguish the two types of foci (Fig. 1f).

Evidence for both transforming and non-transforming virus in stocks of clone purified OK 10 virus

In interference assays performed to define the subgroup of OK 10 virus it was evident that the virus stock contained both transforming and non-transforming virus. Interference against challenge virus, RSV(RAV-1) was obtained about two logs beyond the end point of the transforming titre of OK 10 virus (Table 1). We refer to these viruses henceforth as OK 10 V (transforming virus) and OK 10 AV (associated virus, which is non-transforming); OK 10 virus refers to the complex.

Attempts to separate the transforming virus and the associated virus of the OK 10 virus complex

In order to separate OK 10 V from OK 10 AV the following experiments were performed: the OK 10 AV was isolated by end point titrations. Chick embryo cells were infected with different dilutions of OK 10. Cells from the last dilution showing 50% interference and no foci were passaged several times (Table 1). Virus was collected and stored at -70 °C to serve as OK 10 AV stock. This virus did not cause any foci on CEC in several experiments. However, after several passages it induced the 'conversion' of CEC, as described earlier (Oker-Blom et al. 1975). Intravenous inoculation of OK 10 AV induced leukotic lesions of transformed lymphoblasts in the Bursa of Fabricius, liver, bone marrow,
Fig. 1. Morphological alterations in CEC infected with OK 10 virus and RSV(RAV-1) virus. 
(a) Uninfected CEC monolayers. Magnification ×40. (b) Morphological changes induced by undiluted OK 10 virus on CEC. Magnification ×80. (c) Focus formation by OK 10 virus under agar overlay. Magnification ×40. (d) OK 10 virus focus at a greater magnification (×140). (e) RSV(RAV-1) induced focus, showing focus with empty centre. Magnification ×80. (f) OK 10 virus foci and RSV(RAV-1) foci on the same plate in an interference assay. Magnification ×20.

heart and muscle in two out of 7 chicks after 17 and 19 months (Hortling, 1978). The other chickens are still healthy nearly 2 years later.

Attempts to purify the transforming component OK 10 V from its associated virus were made by infecting CEC with stock virus under agar overlay and by isolating single foci. In one such experiment, 18 out of 20 foci produced both OK 10 AV and OK 10 V. Two foci
Table 1. Capacity of OK 10 virus to interfere with RSV of subgroups A, B, C and D

| Dilution of OK 10 used to infect CEC | Number of OK 10 foci | Capacity of virus from passage 4 of OK 10 infected CEC to interfere with subgroups*:
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>10^-3</td>
<td>233 i.c.†</td>
<td>A 1.19</td>
</tr>
<tr>
<td>10^-4</td>
<td>18 i.c.†</td>
<td>B 1.27</td>
</tr>
<tr>
<td>10^-5</td>
<td>— 0.15†</td>
<td>C 0.92</td>
</tr>
<tr>
<td>10^-6</td>
<td>— 0.99</td>
<td>D 0.92</td>
</tr>
<tr>
<td>10^-7</td>
<td>— 0.92</td>
<td></td>
</tr>
<tr>
<td>10^-8</td>
<td>— 1.01</td>
<td></td>
</tr>
<tr>
<td>10^-9</td>
<td>— 1.05</td>
<td></td>
</tr>
<tr>
<td>10^-10</td>
<td>— 1.20</td>
<td></td>
</tr>
</tbody>
</table>

* The following dilutions were used: RSV(RAV-1) 10^-15, RSV(RAV-2) 10^-8, RSV(RAV-7) 10^-3 and RSV(RAV-50) 10^-3.
† The plates were almost confluent with a mixture of OK 10 and RSV foci, which made it impossible to count (i.e.) challenging virus foci.
‡ Interference is expressed as the relative sensitivity to challenge virus infection of the OK 10 cultures compared to control cultures.

Table 2. Capacity of RSV of subgroups A, B, C and D to interfere with OK 10 virus

| Interfering virus† | RSV (RAV-1)‡ | RSV (RAV-2)‡ | RSV (RAV-7)‡ | RSV (RAV-50)‡ | OK 10§ | OK 10 V§||
|--------------------|--------------|--------------|--------------|--------------|-------|------|
| RAV-1              | +            | —            | —            | —            | +     | +    |
| RAV-2              | —            | +            | —            | —            | —     | —    |
| RAV-49             | —            | —            | +            | —            | —     | —    |
| RAV-50             | —            | —            | —            | +            | —     | —    |
| OK 10 AV           | +            | —            | —            | —            | +     | +    |

* + = Interference; — = no interference.
† Second passage of virus infected chick embryo fibroblasts.
‡ Viruses diluted to 10^-3.
§ Viruses diluted to 10^-1.
|| This virus was isolated from a continuous tumour cell line, which apparently does not contain OK 10 AV (N. Oker-Blom et al. unpublished data).

produced neither virus. Attempts to find transforming virus by superinfection of these cultures with the different RAVs were unsuccessful.

Thus it has not been possible so far to isolate in vitro the transforming component of OK 10 without the associated virus OK 10 AV.

Identification of the subgroup of OK 10 virus

Interference assay

When the interference with transformation in fibroblasts previously infected with various subgroups of avian leukosis viruses was determined, OK 10 proved to be subgroup A (Table 2).

Neutralization test

The neutralization test showed that both OK 10 V and OK 10 AV were of subgroup A. There was no neutralization of OK 10 virus by any antiserum of subgroups B, C or D (Table 3).
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Table 3. Neutralization test for typing of OK 10*

<table>
<thead>
<tr>
<th>Serum</th>
<th>Serum dilution</th>
<th>RSV (RAV-1)†</th>
<th>RSV (RAV-2)†</th>
<th>RSV (RAV-7)†</th>
<th>RSV (RAV-50)†</th>
<th>OK†</th>
<th>OK 10 V†‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-RAV-1 serum</td>
<td>1:100</td>
<td>0.356</td>
<td>0.973</td>
<td>1.0</td>
<td>1.10</td>
<td>0.305</td>
<td>0.487</td>
</tr>
<tr>
<td>Anti-RAV-2 serum</td>
<td>1:100</td>
<td>1.0</td>
<td>0.174</td>
<td>1.0</td>
<td>1.0</td>
<td>0.909</td>
<td>1.0</td>
</tr>
<tr>
<td>Anti-RAV-49 serum</td>
<td>1:100</td>
<td>1.0</td>
<td>0.856</td>
<td>0.579</td>
<td>0.876</td>
<td>1.0</td>
<td>0.986</td>
</tr>
<tr>
<td>Anti-RAV-50 serum</td>
<td>1:20</td>
<td>1.0</td>
<td>0.712</td>
<td>1.0</td>
<td>0.668</td>
<td>1.0</td>
<td>0.939</td>
</tr>
<tr>
<td>Anti-OK 10 serum</td>
<td>1:20</td>
<td>0.011</td>
<td>0.957</td>
<td>1.0</td>
<td>1.0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Anti-OK 10 AV</td>
<td>1:100</td>
<td>0.0</td>
<td>0.769</td>
<td>1.0</td>
<td>0.924</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* The neutralization is calculated as the fraction of virus surviving after neutralization.
† The virus was diluted to $10^{-2.5}$.
‡ This virus was isolated from a continuous tumour cell line, which does not contain OK 10 AV in detectable amounts (N. Oker-Blom et al. unpublished data).

Biochemical properties of OK 10 virus

The budding particles seen in cells in the electron microscope, and the negatively stained particles, are indistinguishable from other C type particles. Purified virus has a density of 1.16 g/ml in sucrose gradients. No difference was seen between high and low titre virus, i.e. OK 10 and OK 10 AV alone. The virus contained 70S RNA and 35S RNA subunits (Fig. 2), and as far as could be judged from gel electrophoresis of heated RNAs, the 35S RNA subunits of OK 10 virus belong to class b (Fig. 2). No differences were detected in the electrophoretic mobilities of the RNAs of the OK 10 virus and OK 10 AV or between their RNAs and the RNAs of RAV-1 or RAV-2. It has to be remembered, however, that the transforming virus represents only about 1% of the total virus in the preparations used in these experiments.

Growth of OK 10 virus infected cells in soft agar

Monolayers of CEC were infected with different dilutions of OK 10 virus and, after one or two passages, the cells were seeded in soft agar. After seven days incubation, three types of colonies were observed. Large colonies consisting of dispersed cells, medium sized fairly compact colonies and very small colonies. RSV (RAV-1) infected cells give both the large disperse type and the compact medium size colonies, BHK cells also give rise to two types of colonies, and uninfected CEC may yield small compact colonies containing up to ten or twenty cells. The very small colonies therefore should be regarded with caution. This may also hold true for the two or three types of colonies formed by different transformed cells, although the possibility that different types of colonies represent different cells or cells infected by different viruses has to be kept in mind. The colony forming efficiency was $6.2 \times 10^2$ colonies in 1 million cells.

Pathogenity of OK 10 virus for chicken

In order to study the effect of the different components of OK 10 virus in chicken, 2 to 4-day-old chicks were injected intravenously with OK 10 or OK 10 AV virus. Of the nine chicks injected intravenously with $6 \times 10^3$, $6 \times 10^2$ and $6 \times 10^1$ f.f.u. of OK 10, 8 came down with an acute leukotic-like disease 1 to 2 months after injection. The only negative was one of three chickens receiving the smallest dose of virus. The lesions were very similar to those described for the MH 2 tumour (Campbell 1969; the histological examination was kindly
Fig. 2. (a) Isolation of 70S RNA from purified OK 10 virus. The virus was treated with 1 ml of 2% SDS in TSE-buffer supplemented with 5 ml of 0.05 M-EDTA, layered on a 15 to 30% (w/w) sucrose gradient, and was centrifuged for 6 h at 22 °C, and 24,000 rev/min, using a Beckman SW rotor. Semliki Forest virus 42S RNA was used as a marker. ●–●, 32P-labelled OK 10 virus; ○–○, 3H-uridine labelled Semliki Forest virus RNA. (b) Gel electrophoresis of 32P-labelled OK 10 virus RNA, using 2% polyacrylamide-0.5% agarose gels containing 0.1% SDS. The RNA was heated for 1 min at 100 °C prior to electrophoresis. The gels were run for 3 h at 200 V (10 V/cm), sliced into 2 mm pieces and treated with 0.7 ml NCS. The radioactivity was determined in a Packard liquid scintillation counter. ○–○, 3H-uridine labelled PR-C RNA; ●–●, 32P-labelled OK 10 virus RNA.

DISCUSSION

Many of the properties of the OK 10 virus described in this paper seem to differ from those of other transforming avian leukosis viruses, such as avian myelomatisis virus (AMV), avian erythroblastosis virus (AEV) and the myelocytomatosis virus MC 29. It has, however, some properties in common with the virus of the MH 2 endothelioma isolated by Begg in 1927 and later described by Payne & Biggs (1970) and should apparently be placed in the group of acute avian leukaemia viruses. These viruses have lately attracted con-
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siderable interest as being probable representatives of the avian carcinoma viruses (Duesberg et al. 1977).

Two possible origins of the virus have to be considered. The more plausible is that the virus represents a field isolate like MC 29 and MH 2. The other possibility is that the transforming agent is an endogenous virus obtained from chickens after stimulation with a non-transforming leukosis virus.

OK 10 virus belongs to subgroup A. Thus, OK 10 also differs from the known acute leukaemia viruses like MC 29, which seems to contain helper viruses of both subgroup A and B, and MH 2, which seems to belong to subgroup C.

The question of the helper dependence of the transforming component of OK 10 virus is open, as is that of virus OK 10 AV which is non-transforming in vitro. This latter virus induced a leukotic disease in two out of seven chickens of the C/O line after 17 and 19 months of incubation. Thus, OK 10 AV resembles the common non-transforming leukosis viruses. Attempts to separate OK 10 V from the complex in vitro have so far been unsuccessful, as have attempts to enhance the titre of OK 10 V by using a helper of any subgroups available.

The pathology of the virus infection in chickens is described in detail in another paper (Hortling, 1978), but it has many features in common with the MH 2 virus. It differs, however, in that it does not cause lesions on the CAM of the fertile eggs of the C/O line and it does not cause local tumours in chicks of the same line if inoculated intramuscularly and/or intra and subcutaneously. When tumour cells induced by the virus in vitro are injected intramuscularly into chicks no local tumours arise. Chicks infected with purified virus either intraperitoneally, intravenously or intramuscularly do, however, succumb within a few weeks to an acute leukaemoid disease with visceral tumours.

Electron microscopy of infected cells as well as the stable line of tumour cells shows budding C-type particles indistinguishable from conventional C-type particles. From such particles 70S RNA can be isolated. This RNA, when heated produces b-type subunits only. Since virus isolated from monolayers of chick embryo cells contains the non-transforming OK 10 AV in excess, it cannot definitely be excluded that a-type subunits are also present. The RNA of OK 10 may thus differ in size from the RNA of RSV. Whether it also differs from the RNA of the non-transforming leukosis viruses, remains to be seen. It may be similar to, for instance, the RNAs of MH 2 and MC 29, which are said to contain smaller RNAs than other C-type viruses. Experiments to study this question have been initiated.

The data so far available indicate that OK 10 virus is related to MH 2 virus, or to the acute leukaemia viruses of chicken. These viruses may turn out to be excellent models for the study of the oncogenes of the transforming avian leukosis viruses and acute leukaemia viruses, along the lines successfully used for study of the oncogenes of the sarcoma viruses (Stehelin et al. 1976a, b).

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REFERENCES


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