Replication of Gross Leukemia Virus in Long-Term Cultures of Rat Thymomas: Bioassays and Electron Microscopy

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Summary

Two cultures of leukemic cells were established from Gross virus-induced rat thymomas and grown in vitro for 29 and 36 months.

A constant and predictable virus yield was obtained that resulted in 100% induction of leukemia when cell-free materials from these cultures were assayed in susceptible animals.

Electron microscopic studies of these cultures confirmed the abundance of virus and permitted observations on formation, release, and phagocytosis of virus particles by the leukemic cells.

Introduction

Since a filterable agent was demonstrated to be the cause of spontaneous leukemia in Ak mice (12), extensive work utilizing different approaches has been undertaken to study leukemia viruses.

To learn about the cells and conditions providing optimal replication, growth of murine leukemia viruses in vitro has been attempted. This would permit subsequent investigations of the morphology, location, and biologic activity of leukemia viruses in culture. A constant and convenient source for viral supply, needed for further research, would also be provided.

A summary of work done thus far with murine leukemia viruses in tissue cultures is given in Table 1. It is seen that different types of cells from mouse tissues supported the growth of leukemia viruses for varying periods of time. In addition, the Graffi virus was grown for up to 40 days in HeLa and in calf kidney cells. In some instances, cultures assayed for virus were still positive after long intervals, although most often the virus yield was low, irregular, and unpredictable (23). So far, only 1 permanent cell line has been established that constantly released a leukemia virus (Graffi virus) over a long period of time (20). Propagation of Rauscher virus in a long-term mixed culture has been recently reported (36). In this case, although high titers of virus have been previously obtained, later bioassays indicated a marked decrease in infectivity. In both studies virus particles were demonstrated in electron micrographs of pellets obtained by high-speed centrifugation of supernatant of cultures.

Materials and Methods

CULTURES. Culture LT1 was originated from a thymic lymphoma induced in a W/Fu rat by Gross Passage A virus (16). Culture LT9 was started from a mediastinal lymphoma produced by i.p. injection of LT1 cells into a young W/Fu rat. Cultures LT1 and LT9 have been grown continuously for 36 and 29 months, respectively, at the time of this report.

A morphologic difference can be noted between these 2 cultures. LT1 is a symbiotic culture of leukemic lymphoblasts floating in the supernatant with a meshwork of reticular cells attached to the glass (Fig. 1). Constant emperipolesis of lymphoid cells, described earlier (16), persisted in this culture (Fig. 1). LT9 is almost totally composed of 1 type of cell that is rounded and floating and has the features of leukemic lymphoblasts (Fig. 2). Both cultures produce mediastinal lymphomas (lymphosarcomas and reticulum cell sarcomas) (Fig. 3) in approximately 14 days in young rats injected i.p. but do not take in rats of the inbred strain that are over 15 days of age. This suggested formation of new cellular antigens (15).

The cultures were grown in Petri dishes placed in a CO2-gassed incubator, in Puck’s medium replaced twice weekly, and were subcultured by scraping every 8–10 days.

ANIMALS. Comparative bioassays of tissue culture material were carried out in W/Fu rats from our randomly bred colony and in 2 kinds of hybrid mice: (AKR/Jax × C3H/Bl)F1 and (AKR/Jax × C3H/Bl)F1 will be abbreviated (AK/C3H/Bl)F1 and (AKR/Jax x C3H/Bl).
**TABLE 1**

**REPORTS ON MURINE LEUKEMIA VIRUSES GROWN IN TISSUE CULTURE**

<table>
<thead>
<tr>
<th>Type of virus</th>
<th>Authors</th>
<th>Time in culture</th>
<th>Bioassays for virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gross Passage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gross</td>
<td>Manaker et al., 1961 (31)</td>
<td>5-6 wk</td>
<td>5-75</td>
</tr>
<tr>
<td></td>
<td>Ginsburg and Sachs, 1962 (10)</td>
<td>290 days</td>
<td>88</td>
</tr>
<tr>
<td>Moloney</td>
<td>Sinkovics et al., 1964 (32)</td>
<td>22 days</td>
<td>4-4</td>
</tr>
<tr>
<td>Graffi</td>
<td>Moore and Friend, 1958 (24)</td>
<td>6 times fluid replaced</td>
<td>not given</td>
</tr>
<tr>
<td>Friend</td>
<td>Sinkovics et al., 1964 (32)</td>
<td>26 days</td>
<td>33-75</td>
</tr>
<tr>
<td>Rauscher</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Authors</th>
<th>Cell type</th>
<th>Time in culture</th>
<th>Bioassays for virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gross et al., 1961 (14)</td>
<td>Mouse embryo</td>
<td>26 days</td>
<td>40 (100 in 1 passage)</td>
</tr>
<tr>
<td>Manaker et al., 1960 (21)</td>
<td>Mouse spleen</td>
<td>1 yr</td>
<td>91</td>
</tr>
<tr>
<td>Manaker et al., 1964 (30)</td>
<td>Mouse spleen</td>
<td>4 yr</td>
<td>Not given</td>
</tr>
<tr>
<td>Ginsburg and Sachs, 1961 (9)</td>
<td>Mouse embryo, mouse kidney</td>
<td>14-39 days</td>
<td>7-75</td>
</tr>
<tr>
<td>Salaman et al., 1961 (30)</td>
<td>Mouse embryo</td>
<td>3 passages</td>
<td>58</td>
</tr>
<tr>
<td>Graffi and Schramm, 1963 (11)</td>
<td>Mouse embryo</td>
<td>45 days</td>
<td>104-267 days</td>
</tr>
<tr>
<td>Schramm and Graffi, 1963 (31)</td>
<td>HeLa</td>
<td>20 days</td>
<td>128 days</td>
</tr>
<tr>
<td>Moore and Friend, 1958 (24)</td>
<td>Mouse embryo</td>
<td>Not given</td>
<td></td>
</tr>
<tr>
<td>Moore, 1969 (22); 1963 (23)</td>
<td>Mouse embryo</td>
<td>448-633 days</td>
<td>20-40 (irregular)</td>
</tr>
<tr>
<td>Chunmarrt et al., 1961 (3)</td>
<td>Mouse embryo</td>
<td>30 days</td>
<td>77</td>
</tr>
<tr>
<td>Wogood et al., 1964 (20)</td>
<td>Mouse embryo</td>
<td>5-15 days</td>
<td>Not given</td>
</tr>
<tr>
<td>Wright and Lasfargues, 1953 (38)</td>
<td>Mouse spleen and thymus</td>
<td>104 days</td>
<td>27 days</td>
</tr>
<tr>
<td>Sinkovics et al., 1964 (32)</td>
<td>Mouse spleen and thymus</td>
<td>7 passages</td>
<td>3-6 mo</td>
</tr>
<tr>
<td>Pereis et al., 1964 (27)</td>
<td>Mouse kidney infected in vivo</td>
<td>7-71 days</td>
<td>85-100 days</td>
</tr>
</tbody>
</table>

Note: On mouse spleen feeder layers.

More recent bioassays have indicated a marked decrease in infectivity.

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An/Hc/Jax)F1 will be abbreviated (AK/C3H/An)F1. Animals 1–6 days of age were injected i.p. using 0.05 ml, for mice, and 0.1 ml, for rats, of the assayed fluids. The litter was usually sacrificed after the 1st animal died with leukemia. Alternate litters were used as controls and kept until natural death. Bioassay results were evaluated by gross and microscopic examination.

Only animals surviving more than 60 days after inoculation were considered.

**Bioassays.** Cultures LT1 and LT2, as well as the mediastinal lymphomas, Ly LT1 and Ly LT2, produced by i.p. injection of the respective cultures in young rats, were assayed separately. Similar cultures were pooled (25–35 ml total quantity of supernatant plus cells) and injected as 1 of the following: (a) cell-containing supernatant (used only for bioassays in mice); (b) frozen-thawed material, i.e., cell-containing supernatant, twice frozen at −20°C and thawed at 37°C; centrifuged 10 min at 2000 rpm in a refrigerated centrifuge; supernatants used for injection; (c) cell-free material, i.e., cell-containing supernatant centrifuged at 1400 × g and 7000 × g each for 20 min at 4°C; supernatants used for injection; (d) cell-free filtrate, i.e., cell-containing supernatant centrifuged at 1400 × g and 7000 × g, as above. Supernatant passed through Selas 0.2 porosity filter candle that retained *Escherichia coli*. Tumors Ly LT1 and Ly LT2 were finely minced, suspended in Phell's medium, and processed as described under (b), (c), or (d).

**Electron Microscopy.** After 4–7 days subcultures were gently scraped with rubber policeman, pooled, and centrifuged for 5 min in a refrigerated centrifuge at 800 rpm. The supernatant was discarded, and the pellet fixed overnight at 4°C in 6% glutaraldehyde according to the method of Sabatini et al. (29). The fixed cells were transferred to a phosphate buffer for 24 hr at 4°C and postfixed in osmic acid for 24 hr at 4°C according to the method of Caulfield (2). Fragments from lymphomas fixed in situ were similarly processed. The fragments were embedded in Epon, and after polymerization were sectioned and placed on bare 300-mesh grids. These were stained at room temperature for 15 min with fresh alcoholic uranyl acetate (4% in 50% ethyl alcohol) and for 10 min at room temperature with lead citrate...
TABLE 2

<table>
<thead>
<tr>
<th>Material</th>
<th>Time in culture (days)</th>
<th>Preparation</th>
<th>L/T</th>
<th>% with leukemia</th>
<th>Av. latency (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LT&lt;sub&gt;1&lt;/sub&gt;</td>
<td>237</td>
<td>Cell-free filtrate</td>
<td>7/7</td>
<td>100</td>
<td>93</td>
</tr>
<tr>
<td>LT&lt;sub&gt;1&lt;/sub&gt;</td>
<td>597</td>
<td>Cell-containing supernatant</td>
<td>5/5</td>
<td>100</td>
<td>84</td>
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<tr>
<td>LT&lt;sub&gt;1&lt;/sub&gt;</td>
<td>907</td>
<td>Cell-free filtrate</td>
<td>13/13</td>
<td>100</td>
<td>96</td>
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<tr>
<td>LT&lt;sub&gt;2&lt;/sub&gt;</td>
<td>460</td>
<td>Frozen-thawed material</td>
<td>7/8</td>
<td>87</td>
<td>93</td>
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<td>LT&lt;sub&gt;3&lt;/sub&gt;</td>
<td>574</td>
<td>Frozen-thawed material</td>
<td>9/9</td>
<td>100</td>
<td>94</td>
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<td>Lymphoma</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Ly LT&lt;sub&gt;1&lt;/sub&gt;/1</td>
<td>130&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Cell-containing supernatant</td>
<td>6/6</td>
<td>100</td>
<td>97</td>
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<tr>
<td>Ly LT&lt;sub&gt;1&lt;/sub&gt;/2</td>
<td>778</td>
<td>Frozen-thawed material</td>
<td>5/6</td>
<td>83</td>
<td>83</td>
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<tr>
<td>Ly LT&lt;sub&gt;2&lt;/sub&gt;/1</td>
<td>492</td>
<td>Cell-free material</td>
<td>9/11</td>
<td>81</td>
<td>118</td>
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<td>Ly LT&lt;sub&gt;2&lt;/sub&gt;/2</td>
<td>742</td>
<td>Cell-free filtrate</td>
<td>9/9</td>
<td>100</td>
<td>92</td>
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</tbody>
</table>

<sup>a</sup> Out of 129 (AK/C3H/Bi)F₁ mice kept as controls, 33 died with leukemia at 236-330 days of age; 90 from 240-350 days of age were alive and healthy.

<sup>b</sup> L, number of mice that died with leukemia; T, total number of inoculated mice surviving more than 60 days.

<sup>c</sup> Age of the culture from which the lymphoma was derived.

TABLE 3

<table>
<thead>
<tr>
<th>Material</th>
<th>Time in culture (days)</th>
<th>Preparation</th>
<th>L/T</th>
<th>% with leukemia</th>
<th>Time when sacrificed (days)&lt;sup&gt;b&lt;/sup&gt;</th>
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</thead>
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<tr>
<td>Culture</td>
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<td></td>
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<td></td>
<td></td>
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<td>LT&lt;sub&gt;1&lt;/sub&gt;</td>
<td>370</td>
<td>Cell-containing supernatant</td>
<td>0/4</td>
<td>0</td>
<td>135</td>
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<tr>
<td>LT&lt;sub&gt;2&lt;/sub&gt;</td>
<td>630</td>
<td>Frozen-thawed material</td>
<td>0/8</td>
<td>0</td>
<td>150</td>
</tr>
<tr>
<td>LT&lt;sub&gt;1&lt;/sub&gt;</td>
<td>425</td>
<td>Frozen-thawed material</td>
<td>0/12</td>
<td>0</td>
<td>180</td>
</tr>
<tr>
<td>LT&lt;sub&gt;2&lt;/sub&gt;</td>
<td>502</td>
<td>Cell-containing supernatant</td>
<td>0/4</td>
<td>0</td>
<td>180</td>
</tr>
<tr>
<td>Lymphoma: Ly LT&lt;sub&gt;3&lt;/sub&gt;/3</td>
<td>378&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Frozen-thawed material</td>
<td>0/15</td>
<td>0</td>
<td>180</td>
</tr>
</tbody>
</table>

<sup>a</sup> L, number of mice that died with leukemia; T, total number of inoculated mice surviving more than 60 days.

<sup>b</sup> Days after inoculation.

<sup>c</sup> Age of the culture from which the lymphoma was derived.

TABLE 4

<table>
<thead>
<tr>
<th>Material</th>
<th>Time in culture (days)</th>
<th>Preparation</th>
<th>L/T</th>
<th>% with leukemia</th>
<th>Time when sacrificed (days)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>LT&lt;sub&gt;1&lt;/sub&gt;</td>
<td>181</td>
<td>Cell-free filtrate</td>
<td>1/6</td>
<td>16</td>
<td>140</td>
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<tr>
<td>LT&lt;sub&gt;1&lt;/sub&gt;</td>
<td>580</td>
<td>Cell-free filtrate</td>
<td>1/9</td>
<td>11</td>
<td>180</td>
</tr>
<tr>
<td>LT&lt;sub&gt;2&lt;/sub&gt;</td>
<td>704</td>
<td>Frozen-thawed material</td>
<td>0/8</td>
<td>0</td>
<td>200</td>
</tr>
<tr>
<td>LT&lt;sub&gt;2&lt;/sub&gt;</td>
<td>380</td>
<td>Frozen-thawed material</td>
<td>0/13</td>
<td>0</td>
<td>180</td>
</tr>
<tr>
<td>LT&lt;sub&gt;2&lt;/sub&gt;</td>
<td>507</td>
<td>Frozen-thawed material</td>
<td>0/8</td>
<td>0</td>
<td>140</td>
</tr>
</tbody>
</table>

<sup>a</sup> L, number of rats that died with leukemia; T, total number of inoculated rats surviving more than 60 days.

<sup>b</sup> Days after inoculation. The latency period was from 94-110 days.

Results

Bioassays. Cultures LT<sub>1</sub> and LT<sub>2</sub> were bioassayed at intervals from 237 to 907 days of growth in vitro. Viral-induced leukemia was obtained in 97% of the inoculated (AK/C3H/Bi)F₁ mice, with an average latency period of 93 days (Table 2).

Similarly, bioassays were carried out with lymphomas (Ly LT<sub>1</sub>/1, Ly LT<sub>1</sub>/2, Ly LT<sub>2</sub>/1, Ly LT<sub>2</sub>/2) produced in young rats by i.p. injections of cells from the 2 cultures after 130-778 days of growth. The bioassayed (AK/C3H/Bi)F₁ mice were positive for leukemia virus in 90-90% of the cases with an average latency period of 94 days (Table 2).

Spontaneous leukemia in the 129 (AK/C3H/Bi)F₁ mice kept as controls has occurred in 33 thus far, at 236-330 days of age. All the others from 240-360 days of age were alive and apparently healthy. (AK/C3H/Bi)F₁ mice kept as controls in previous experiments had spontaneous leukemia in 73% with latency periods of 240-600 days (19). In absence of leukemia virus, tissue culture material (cells and supernatant) did not accelerate spontaneous leukemia in these hybrid mice (128 mice in other experiments were all apparently healthy at 5-7 months after inoculation).

In the present experiments, both cell-containing and cell-free materials from cultures and derived tumors were similarly effective. Lymphomas produced in mice by cell-containing materials were examined for origin by grafting fragments in newborn rats. The grafts took only in mice, confirming that the grafts were composed of mouse cells from the donors with viral-induced leukemia. As the cultures were of rat origin, this was a precaution to eliminate eventual heterografts in newborn mice. Bioassays of both cultures at different ages and of derived tumors were consistently negative with (AK/C3H/An)F₁ mice (Table 3).

When newborn rats were used to test the presence of virus in the cultures, the percentage of animals positive for leukemia was 100. The average latency period was 70 days in rats and 90 days in mice (Table 5).

Viral-induced leukemia in mice appeared grossly at autopsy as a systemic disease involving thymus, lymph nodes, and spleen, all considerably enlarged by lymphomatous growth (Fig. 4). In rats, only the thymus was replaced by a huge lymphoma, causing death of the animal.

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from that in vivo. A tumor seems to accumulate much of the
destruction among the debris.

in the case of leukemia induction), whereas a culture contains
virus produced during the latency period (an average of 100 days
venient source of virus that can be easily stored in the frozen

Discussion

The histologic type varied between pure and mixed lympho-
cytic and reticulum-cell sarcomas.

ELECTRON MICROSCOPY. Both cultures and derived lymphomas
contained abundant virus particles (Figs. 5, 6). From the sam-
ping made, it appeared that sometimes more virus was present
in the cultures than in the tumors. In a number of cases, thymic
cells from the cultures were surrounded by hundreds of virus
particles. In general, they were extracellular and had an electron-
dense center and an average diameter of 1000 Å. They were iden-
tified with the Type C particle characteristically associated with
viral leukemias (1, 5).

In relation to the cells, virus was observed in both reticular
cells and leukemic lymphoblasts.

Intracytoplasmic vesicles and the plasma membrane were the
2 major sites where virus particles were located in the cell. The
vesicles usually were peripheral and adjacent to the plasma
membrane and frequently protruded in the form of blebs (Figs.
14–16, 18). Virus particles were present, free in the vesicles (Figs.
13–16, 18) and occasionally budding from the inner surface
(Fig. 12), suggesting maturation of the virus at this site. Vesicles
protruding at cellular surfaces were particularly frequent in
culture LT8, and multiple blebs often appeared on the same cell
(Fig. 15). Virus particles at the cell surface were seen as localized,
electron-dense buds (Figs. 8, 11) as projections at the tip of a
stalk (Figs. 7, 9), or as free particles along the plasma membrane
(Figs. 9, 10, 17). Frequently, cellular processes partially sur-
rounded groups of virus particles (Figs. 14, 17–20).

The largest concentration of virus was found in areas of cellular
destruction among the debris.

TABLE 5
Comparative Bioassay in Rats and Hybrid Mice of
Concentrated Rat-adapted Gross Leukemia

<table>
<thead>
<tr>
<th>Recipient</th>
<th>L/T*</th>
<th>%</th>
<th>Av. latency (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>W/Fu rats</td>
<td>3/3</td>
<td>100</td>
<td>70</td>
</tr>
<tr>
<td>(AK/C3H/Bi)F1 mice</td>
<td>6/6</td>
<td>100</td>
<td>82</td>
</tr>
<tr>
<td>(AK/C3H/Bi)F2 mice</td>
<td>10/10</td>
<td>100</td>
<td>96</td>
</tr>
</tbody>
</table>

* L, number of animals that died with leukemia; T, total number of animals inoculated surviving more than 60 days.

In rats, nonconcentrated virus obtained from cultures induced
a low incidence of leukemia. When concentrated, rat-adapted
virus obtained from rat lymphomas was used, there was a 100%
incidence of leukemia in both W/Fu rats and (AK/C3H/Bi)F1
mice. The average latency period was even shorter in rats than in
mice. This difference in susceptibility to the 2 kinds of virus may
be explained on the basis of virus concentration. Nonconcentrated
virus obtained from cultures, although adequate for the highly
sensitive (AK/C3H/Bi)F1 mouse, was perhaps insufficient to
induce leukemia in rats. Another possible explanation is that
successive passages of the virus in rats enhanced its potency for
this recipient. This specific affinity may then disappear during
prolonged cultivation in vivo. A certain host specificity as a
transient quality acquired by a virus through subsequent pas-
sages was recently suggested (34).

The leukemic cell seems to be a propitious site for continuous
replication of the initial virus. In addition to the highly positive
bioassays, electron microscopic examinations confirmed the
abundance of virus in these cultures. At sampling, it even
appeared that more virus was present in the cultures than in the
tumors. Considering that the cultures are free of eventual inhibi-
tors present in the animal (17), the observation is not surprising
and agrees with similar conclusions regarding other viruses.
This is worth considering in view of the rapid growth of leukemic
cells in these cultures, thereby providing a means to obtain large
amounts of virus.

The greatest amount of virus particles was found extracellu-
larly, particularly among the debris of destroyed cells. This
may be due to a cytopathic effect of the virus with the release of

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virus particles upon cellular breakdown. The preferential location of virus particles in areas of cellular necrosis has also been noted with lymphomas (5, 7, 18, 25). Most of the virus particles observed in the extracellular spaces have the features attributed to the mature form of the leukemia virus (5, 7).

In observing virus-cell relationships in this material, maturation of virus seemed to come about in the following sequence. The virus particle first becomes visible as a localized electron-dense projection at the cellular surface (Fig. 11). It undergoes further development, maintaining contact with the cell by means of a stalk (Fig. 7). The stalk elongates (Fig. 8, 9) and finally breaks away completely (Fig. 10). This sequence of events is similar to that previously described as occurring in lymphomas induced by different leukemia viruses (5-7, 25).

A peculiar observation in these cultures was that of numerous protruding cellular vesicles containing a variable number of virus particles. Sometimes, the particles seemed to bud from the inner membrane of the vesicle, to accumulate in the empty space, and finally to be released by the breakdown of the vesicle walls. It is accepted that morphogenesis of RNA viruses takes place in the cytoplasm and that the final stages of virus maturation occur at membranous surfaces of the host cell (4). It appears from some of the present observations that in addition to the plasma membrane, the membrane of cytoplasmic vesicles may be a site for virus maturation. Formation of virus particles and development to mature forms (virions) may follow different patterns in lymphocytic and reticular (epithelial) cells, as suggested by De Harven (6).

Often, cells appeared with thin, elongated processes which partially or totally surrounded clusters of virus particles adjacent to the cell surface. These processes seemed to be involved in cellular engulfment of virus particles. Incorporation of virus by the host cell takes place as a nonspecific process of phagocytosis from the cell surface. These processes seemed to be involved in cellular engulfment of virus particles. Incorporation of virus by the host cell takes place as a nonspecific process of phagocytosis.

Systematic studies are in order to discern what conditions favor formation, release, and phagocytosis of virus. The specific role of lymphoid and reticular cells in release and engulfment of virus particles should also be ascertained. Such studies of the interrelationship of virus and cell at the ultrastructural level can be undertaken with established cultures of virus-producing leukemic cells. In the absence of the alien cells of the feeder layers, these cultures, growing under steady conditions, are also suitable for quantitative studies on virus and cell growth in vitro.

Finally, a culture in which leukemia virus is constantly released by living cells could be used with greater advantage in parabiotic or microdiffusion cultures to study over long intervals the effects of leukemia virus on other types of cells.

Acknowledgment

The technical help of Miss Marlene Sabbath in the tissue culture work is gratefully acknowledged.

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References


22. Moore, A. E. Effect of Carboxypeptidase on Extracts of the Gross Leukemia Virus in Culture
Harry L. Ioachim, Leonard Berwick, and Jacob Furth


Fig. 1. Culture LT1 (929 days in vitro). Reticular cells—dark, spread out, forming a network in the background—and leukemic lymphoblasts—rounded, refringent, mostly floating. In the center, a large reticular cell displays below its nucleolated nucleus a lymphoblast (dark) within a vacuole (clear halo)—emperipolisis. Phase contrast microscope. X 160.

Fig. 2. Culture LT3 (742 days in vitro). Leukemic lymphoblasts—rounded, refringent, mostly floating in the supernatant. Phase contrast microscope. X 160.

Fig. 3. Reticieulum-cell sarcoma (mediastinal), Ly LTj/1, produced in a 10-day-old W/Fu rat, 12 days after i.p. inoculation of cells from culture LT1. X 480.

Fig. 4. Generalized leukemia in an (AK/C3H/Bi)F1 mouse 96 days after i.p. injection of cell-free filtrate of culture LT1 (907 days in vitro). The lymphomatous growth involves thymus; spleen; and mediastinal, mesenteric, and peripheral lymph nodes.

Fig. 5. Culture LT1 (781 days in vitro). A partially formed virus particle budding from the surface of a cell to which it is still attached by a well-defined stalk. A mature particle (virion) lies in the extracellular space. X 28,000.

Fig. 6. Lymphoma Ly LTj/1. A budding particle attached to a cell by a very thin stalk. The thickened membrane at the cell surface (top of the picture) probably represents an early stage of this budding process. X 58,500.

Fig. 7. Lymphoma Ly LTj/1. Two virus particles at the surface of a cell. No attachment seems to be present for the lower particle. The upper particle still has the fuzzy remnants of its stalk. X 58,500.

Fig. 8. Lymphoma Ly LTj/1. Three complete virus particles (virions) within a vesicle of a lymphomatous cell. X 58,500.

Fig. 9. Lymphoma Ly LTj/1. Three complete virus particles (virions) within a vesicle of a lymphomatous cell. X 58,500.

Fig. 10. Lymphoma Ly LTj/1. A fully formed particle completely separated from its lymphoid host cell. The cell still shows a protruberance at the site of what probably was the stalk attachment. X 58,500.

Fig. 11. Lymphoma Ly LTj/1. A partially formed virus particle budding from the surface of a cell to which it is still attached by a well-defined stalk. A mature particle (virion) lies in the extracellular space. X 28,000.

Fig. 12. Leukemic lymphoblast displaying intracytoplasmic vesicles: one next to the plasma membrane and numerous associated virus particles. X 28,000.

Fig. 13. Lymphoma Ly LTj/1. A fully formed particle completely separated from its lymphoid host cell. The cell still shows a protruberance at the site of what probably was the stalk attachment. X 58,500.
Replication of Gross Leukemia Virus in Long-Term Cultures of Rat Thymomas: Bioassays and Electron Microscopy

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