Summary

Four lines of transplantable tumors, WFT-1, WFT-2, WFT-3, and WFT-4, were obtained in Wistar-King-Aptekman/Mk rats after the Friend leukemogenic virus was injected at birth. Histologically, WFT-1, WFT-3, and WFT-4 were lymphosarcomas. WFT-1 showed the "starry sky" picture described in Burkitt's African lymphoma. WFT-2 appeared to be a reticulum cell sarcoma. Both virus particles and budding were observed in these tumors by electron microscopy. It was demonstrated that these tumors will proliferate in rats if conditioned by injection of the Friend virus when they are neonates, or if they are treated with immunosuppressive procedures. However, these tumors failed to grow progressively in nonconditioned isologous rats. The mechanism of these phenomena are discussed.

Introduction

Attempts have been made to induce neoplastic changes and to obtain transplantable tumors in nonsusceptible species by injecting oncogenic viruses into newborn animals (7, 10, 11). Recovery of the virus from such virus-induced neoplastic cells follows a different pattern from that observed in the cells of normally susceptible animals (5). The role of the virus in the maintenance and proliferation of virus-induced neoplastic cells is still uncertain and requires further investigation (8).

Materials and Methods

Friend Virus

The Friend virus was obtained from Dr. Tadashi Yamamoto, Institute of Medical Science, Tokyo University, Japan, and has been serially maintained in dd/Om mice by the transfer of spleen homogenates. Usually, 0.3 ml Friend virus suspension (titered with $10^3-10^4$ ID$_{50}$/ml), recovered from the spleen by Chenaille's method (2), was inoculated intraperitoneally and subcutaneously into rats within 48 hours after birth.

Conditioning Materials

The most effective conditioning material, and the one most frequently used, was the live Friend virus (usually 0.3 ml Friend virus suspension with $10^3-10^4$ ID$_{50}$/ml). Inactivated materials were also used for some of the experiments. The inactivated virus was made by placing the live virus into diluted formaldehyde-Locke's solution (38% formaldehyde diluted 500-fold by Locke's solution) for three weeks in a cold room. During this time the virus preparation was shaken every other day. The inoculum of inactivated virus injected into newborn rats was the same as that of the live virus (0.3 ml). The nonvirus-producing (NVP) type of Friend tumor cells ($5 \times 10^6$) was inactivated in the same manner as the virus preparation and was also injected intraperitoneally and subcutaneously into newborn rats. This NVP cell line has been described by Kobayashi et al. (9).

Rats

Male and female Wistar-King-Aptekman/Mk rats were used. They were bred at the Laboratory for the Breeding of Experimental Animals, Hokkaido University, Sapporo.

Electron Microscopic Examination

To prepare for electron microscopic examination, the solid and ascites forms of tumors were fixed in 1% OsO$_4$ solutions, buffered at pH 7.3, and dehydrated by routine methods. After dehydration, the specimen was embedded in epoxy resin. Ultrathin sections were made by a LKB-II microtome equipped with glass knives. The sections were stained with uranium acetate and lead hydroxide. A Transcope-80 (Akashi) electron microscope was then used.

Tissue Homogenate

A subcutaneous tumor was surgically removed and minced by scissors. Then Locke's solution was added (5 to 10-fold by volume) and the material ground by the Potter Elvehjem homogenizer. In order to test for the presence of virus, the supernatant was centrifuged at 10,000 X g for 30 minutes, and the homogenized material was inoculated into the peritoneal cavity of Friend virus-susceptible dd/Om mice. Spleen weight was calculated by Moore's method (12) 21 days after injection of the homogenate. Spleen weight below 0.2 gm was referred to as negative, over 0.6 gm as positive, and from 0.2 to 0.6 gm as
“suspicious positive” for the existence of infectious Friend virus.

RESULTS

Establishment and Morphology of the Rat Friend Tumors

The transplantable Friend virus-induced tumors now available in our laboratory are generally referred to as Rat Friend Tumors (RFT). The four lines carried in the Wistar rats, designated as WFT-1, WFT-2, WFT-3, and WFT-4, were derived from 4 of 19 rats that had been injected with the Friend virus at birth.

WFT-1 cells were first obtained from the enlarged spleen of a 244-day-old Wistar-King-Aptekman rat injected with Friend virus at birth; WFT-2 cells, from the enlarged thymus of a 269-day-old Wistar-King-Aptekman rat injected with Friend virus at birth; WFT-3 cells, from the mixture of enlarged thymus and spleen of a 288-day-old Wistar-King-Aptekman rat, so that the precise source of the tumor is not clear; and WFT-4 cells from the enlarged thymus of a 240-day-old Wistar-King-Aptekman rat.

Histologically, WFT-1 resembles a lymphosarcoma, and it shows the “starry sky” picture which is often associated with the African lymphoma, described by Burkitt (1) and O’Conor (16) (Figs. 1, 2). Slender reticulum fibers, a feature characteristic of reticulum cell sarcoma, were also noted in the WFT-1 tumor. Electron microscopy showed relatively large nuclei, a few round mitochondria, poorly developed endoplasmic reticulum, and a number of free ribosomes, thus suggesting that it was a lymphosarcoma (Fig. 3).

The WFT-2 tumor resembles WFT-1, but it shows more variation in cell size and tissue organization. A pattern resembling that of meningioma was often recognized in the late stages of the transplantation, but this appeared to result from central degeneration in whorl-like clusters of small cells. In the early stages of transplantation, this degeneration was observed only rarely (Figs. 4, 5). Development of reticulum fibers was not observed. With the electron microscope, the nuclei were seen to be of various sizes; some were kidney-shaped (Fig. 6).

WFT-3 and WFT-4 appear to be more typical lymphosarcomas. They consist of small round cells with dense, stained nuclei (Figs. 7, 9). Electron microscopy showed a few mitochondria and a poorly developed endoplasmic reticulum (Figs. 8, 10).

In all these tumor lines, growth occurred at the site of subcutaneous transplantation, and regional lymph nodes and distant organs were often involved. WFT-1, WFT-2, and WFT-4 were maintained only in solid form, and they have not been converted to the ascites form so far. WFT-3 was maintained in both solid and ascites form, the ascites form being referred to as WFT-3As. The period between transplant generations was approximately four weeks in the WFT-1A, WFT-3, and WFT-4 lines, and two weeks in the WFT-1B, WFT-2, and WFT-3As lines. WFT-1B is a subline of WFT-1A and was derived from the original line of WFT-1 at the third transplantable passage.

Transplantation to Virus-conditioned and Nonconditioned Rats

RFT cells proliferated in isologous rats conditioned with Friend virus at birth (Friend virus-conditioned rats), but the cells did not proliferate in isologous untreated rats that had not been conditioned with Friend virus at birth (nonconditioned rats) (Table 1). In Friend virus-conditioned rats, the following died of tumor growth: 41 out of the 42 rats inoculated subcutaneously with trocar volume of WFT-1A; all rats inoculated with WFT-1B tumor; 71 out of the 73 rats inoculated with WFT-2 tumor; all 18 rats inoculated with WFT-3 tumor; all 5 rats inoculated with WFT-4 tumor; and all 28 rats inoculated intraperitoneally with WFT-3A tumor. By contrast, none of the 19 nonconditioned rats inoculated with WFT-1A, none of the 4 rats inoculated with WFT-3, none of the 11 rats inoculated with WFT-3As, and none of the 4 rats inoculated with WFT-4 died of tumor growth. However, 2 of the 13 rats in the WFT-1B line and 36 of the 47 rats in the WFT-2 series showed that temporary growth of the tumor reached a maximum of 1.6 x 3.0 cm during the 12th–15th day, and this was followed by regression. Mean survival time, when transplants were made to the Friend virus-conditioned rats, was 18.6 days when the WFT-3As rat tumor was used, which was the shortest, and 65.0 days when the WFT-4 rat tumor was used, which was the longest survival time. The growth rate of the WFT-2 and WFT-3A rat tumors was faster than that of the others. It appeared that the virus tolerance of

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Form</th>
<th>Friend virus-conditioned rats</th>
<th>Nonconditioned normal rats</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Lethal growth</td>
<td>Average survival (days)</td>
</tr>
<tr>
<td>WFT-1A</td>
<td>Solid</td>
<td>41/42</td>
<td>52.1 (22–81)*</td>
</tr>
<tr>
<td>WFT-1B</td>
<td>Solid</td>
<td>58/58</td>
<td>27.8 (12–61)</td>
</tr>
<tr>
<td>WFT-2</td>
<td>Solid</td>
<td>71/73</td>
<td>22.4 (9–54)</td>
</tr>
<tr>
<td>WFT-3</td>
<td>Solid</td>
<td>18/18</td>
<td>45.0 (26–60)</td>
</tr>
<tr>
<td>WFT-3As</td>
<td>Ascites</td>
<td>28/28b</td>
<td>18.6 (11–54)</td>
</tr>
<tr>
<td>WFT-4</td>
<td>Solid</td>
<td>5/5</td>
<td>65.0 (46–80)</td>
</tr>
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</table>

Subcutaneous transplantation of Rat Friend Tumors into isologous Wistar-King-Aptekman/Mk rats.

*Range is indicated in parentheses.

bInoculated intraperitoneally.

cFive out of the 11 rats were inoculated subcutaneously, and the temporary growth was described.
the host required for the growth of RFT differs with the line used.

Relationship between the Friend Virus and Growth of RFT Cells

In order to be certain of the specificity of dependence of growth upon the presence of virus, it was necessary to determine whether the proliferation of RFT cells would occur only in the rats conditioned by the live Friend virus, or whether the growth might take place in rats that received a nonspecific immunosuppressive treatment (Table 2). It is known that tumors will grow in irradiated animals but not in normal animals; therefore, either four rats were X-irradiated with 200 R when 4 days old, or five rats were X-irradiated with 300 R when 32 days old. Then they were inoculated with WFT-1 tissue, but no growth occurred. However, all four adult rats irradiated with 500 R died of tumor growth. It is also known that newborn animals are lacking in immunologic resistance and that tumors may grow in the neonate, but not in the adult rat. Therefore, 8 newborn rats were injected with WFT-1 tissue. Only one died from tumor growth on the 22nd day, and no temporary growth of tumor was seen in the 7 remaining rats. When the WFT-2 tumor was transplanted into 8 newborn rats, all died from tumor growth in approximately 18 days. This result indicates that the tumors grow in newborn and irradiated animals and that this phenomenon is not limited to virus-conditioned rats. However, it seems that previous treatment of the newborn rat by the live virus was more effective in producing successful transplantation, for it lessened defense against the growth of the transplanted tumor even more than nonspecific immunosuppressive measures.

A further test was to inject formalin-treated inactivated materials into newborn rats. Five out of 7 rats in the WFT-1 series and 2 out of 3 rats in the WFT-2 series showed temporary growth of the tumor. When the rats were conditioned with the formalin-treated nonvirus producing (NVP) type of Friend tumor cells of mice, 1 out of 3 rats in the WFT-2 series showed lethal growth, and the two remaining rats showed temporary growth of the tumor. Thus it appears that the effects on tumor growth produced by the formalin-treated virus- or NVP-type cell materials are only temporary and are less efficient than the effects of the live Friend virus. However, it seems that injection of the inactivated materials is equivalent to or more effective than X-irradiation on the newborn state when the WFT-1 rat tumor was used.

Friend Virus in the RFT Cell

By electron microscopic examinations, Friend virus particles were observed in intercellular spaces, and budding particles from the cell membrane were also observed in the RFT cell grown in the virus-conditioned rats. Furthermore, definite virus particles and budding have also been infrequently observed in the WFT-2 cell, which was serially transplanted into virus-nonconditioned rats.

Attempts were made to obtain the infectious Friend virus from the RFT cells. Spleen weight was measured in dd/Om mice which were injected intraperitoneally with homogenates of WFT-2 tissue. As was shown in Table 3, slight enlargement of the spleen was observed in mice injected with the tumor

<table>
<thead>
<tr>
<th>Condition of rats</th>
<th>WFT-1B tumor</th>
<th>WFT-2 tumor</th>
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<tbody>
<tr>
<td></td>
<td>Lethal</td>
<td>Temporary</td>
</tr>
<tr>
<td>Total-body irradiation (R)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>0/4</td>
<td>0/4</td>
</tr>
<tr>
<td>300</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>500</td>
<td>4/4</td>
<td></td>
</tr>
<tr>
<td>Newborn state, 24 hours after birth</td>
<td>1/8</td>
<td>0/7</td>
</tr>
<tr>
<td>Injection of inactivated Friend virus at birth</td>
<td>0/7</td>
<td>5/7</td>
</tr>
<tr>
<td>Injection of inactivated mouse NVP-type Friend tumor cells at birth</td>
<td>1/5</td>
<td>4/4</td>
</tr>
</tbody>
</table>

Growth of Rat Friend Tumor cells in isologous Wistar-King-Aptekman/Mk conditioned rats. NVP, nonvirus-producing.

<table>
<thead>
<tr>
<th>Homogenate obtained from:</th>
<th>Titration</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>10^0</td>
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<tr>
<td>Tumors grown in Friend virus-conditioned rats</td>
<td>0.53</td>
</tr>
<tr>
<td>(0.16-1.45)*</td>
<td>(0.11-1.25)</td>
</tr>
<tr>
<td>Tumors transplanted into nonconditioned rats for 4 generations</td>
<td>0.14</td>
</tr>
<tr>
<td>(0.11-0.19)</td>
<td>(0.10-0.25)</td>
</tr>
<tr>
<td>Physiologic saline solution</td>
<td>0.11</td>
</tr>
<tr>
<td>(0.09-0.14)</td>
<td></td>
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</table>

Recovery of Friend virus from WFT-2. Spleen weight of 5 mice of each group at the 21st day after the injection of homogenates. *Average spleen weight (gm); the range is given in parentheses.
homogenate derived from virus-conditioned rats, but no definite enlargement of the spleen was observed in mice injected with the tumor homogenate derived from virus-nonconditioned rats. This indicates that most of the virus particles may have been derived from the virus used as conditioning material in the virus-conditioned rat and may not have been derived from the virus produced from the RFT cell. In any event, it can be said that the production of the virus is evidently less in the RFT (WFT-2) cells than in most of the Friend tumor cell lines in mice.

**DISCUSSION**

Various researchers working with Friend virus have reported the successful production of transplantable tumors in mice, which may be considered histologically as erythroblastic or reticular cell neoplasms (6, 15). In contrast, it has been reported that the neoplastic lesions in rats inoculated with Friend virus at birth showed a picture of lymphosarcoma or reticulum cell sarcoma (4, 10, 11). The authors, in an attempt to produce transplantable tumors in rats, failed in all 12 cases when virus-nontolerant rats were used; in the present work, success was seen in 4 out of 7 cases when virus-tolerant rats were used. The tumor lesions in rats were found approximately 200 days after the inoculation of the virus, and histologically there was no doubt as to their neoplastic nature (4). Contrary to the cases of Friend disease in mice, the production of infectious virus from the RFT cell was insignificant, and, as a result, no early deaths arising from viremia appeared. Thus we are of the opinion that, if virus-tolerant rats are used, the establishment of RFT will not be too difficult.

The sustained growth of virus-induced tumors in virus-tolerant hosts has been reported previously in several papers (3, 13, 14, 18, 19). For instance, Morton (13) observed that the growth of mammary tumor cells produced by the milk factor may be seen in histocompatible and milk-factor (+) mice, while in milk-factor (−) mice it may not be readily seen. Silobrcic and Suit (19) made a similar report. Recently, Nowinski et al. (14) reported that mouse leukemia induced in ML antigen (+) of mice showed a favorable growth in isologous mice of MTV (+), while in mice with ML antigen (−) it did not. Also, Geering et al. (3) reported that the W/Fu rat leukemia induced by murine leukemia virus showed a growth in W/Fu rats less than 7 days old, while it was difficult to grow these tumors in adult rats. This seems to suggest that the growth of these virus-induced tumors requires a tolerance to virus in the host, similar to that observed in the experiments reported here.

**ACKNOWLEDGMENTS**

We thank Dr. T. B. Dunn and Dr. A. S. Rabson, National Cancer Institute, NIH, Bethesda, Maryland, for their encouragement and cooperation.

**REFERENCES**

Fig. 1. WFT-1 tumor line. Note homogeneous, large, round cells with many phagocytic histiocytes, resembling the Burkitt African lymphoma. H & E, × 360.

Fig. 2. High magnification of the WFT-1 tumor, showing large cells forming the “starry sky” picture. H & E, × 920.

Fig. 3. Electron photomicrograph of the WFT-1 cell with poorly developed mitochondria and endoplasmic reticulum. Epon embedding, × 12,000.

Fig. 4. WFT-2 tumor line. A characteristic field with a whorl-like structure resembling meningioma, probably caused by central degeneration. H & E, × 360.

Fig. 5. WFT-2 tumor line. Note homogeneous round cells. H & E, × 260.

Fig. 6. Electron photomicrograph of the WFT-2 cell transplanted into the nonconditioned rat. Well-developed mitochondria and concave nuclei are seen. × 9,000.

Fig. 7. WFT-3 line. Note homogeneous, round cells. H & E, × 360.

Fig. 8. Electron photomicrograph of WFT-3As tumor cells. Note the concave nuclei and poorly developed mitochondria. × 50,000.

Fig. 9. High magnification of WFT-4 tumor cells. H & E, × 420.

Fig. 10. Electron photomicrograph of the WFT-4 cell with poorly developed mitochondria and endoplasmic reticulum. Virus particles are observed intercellularly and intracellularly. × 8,000.
Transplantable Friend Virus-induced Tumors in Rats


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