Biological and Morphological Studies of SJL/J Strain Reticulum Cell Neoplasms Induced and Transmitted Serially in Low-Leukemia-Strain Mice

Susumu Fujinaga, William E. Poel, W. Clydell Williams, and Leon Dmochowski

Department of Virology, The University of Texas M. D. Anderson Hospital and Tumor Institute at Houston, Texas 77025

SUMMARY

Reticulum cell neoplasms (RCN) were induced in 4 of 34 BALB/c low-leukemia-strain mice inoculated i.p. with supernatants of centrifuged extracts of spontaneous reticulum cell sarcomas of SJL/J strain mice. The extracts were obtained from tumors and from tumorous spleen tissue culture derived from SJL/J strain mice. The RCN's induced in BALB/c mice were transmitted serially to other low-leukemia-strain mice (BALB/c, C3H/f, and C3HeB/FeJ) by similarly prepared cell extracts and by cell-free filtrates of tumorous organs of the mice. The lesions induced included RCN types A and B (Dunn's nomenclature) and heterotopic myelopoiesis, including some cases which histologically could be described as myeloid and erythroid leukemias and megakaryocytosis. The incidence of induced RCN in mice increased to 92%, and the average latent period decreased to 90 days as serial transmission of the RCN progressed.

Electron microscopic examination of lymph nodes, spleen, thymus gland, liver, kidneys, lungs, and bone marrow of mice with induced RCN revealed varying numbers of virus particles irrespective of the histological type of lesions. The virus particles were morphologically identical with murine leukemia type C virus particles and with virus particles observed in spontaneous RCN of SJL/J strain mice. Budding particles were observed in cells of all organs examined. Intracisternal type A virus particles were found in some organs. Type C virus particles were observed in cells of all examined tissue culture passages of tumorous spleen derived from an SJL/J strain mouse with spontaneous RCN. Budding virus particles were also observed in tissue culture, indicating that the particles multiply for prolonged periods in vitro. Biological activity of the virus produced in vitro was demonstrated by induction of RCN in BALB/c strain mice inoculated with supernatants from a centrifuged extract of tissue culture cells. Tissue culture from embryos of an apparently normal SJL/J strain mouse also contained type C virus particles. This indicates vertical transmission of the virus in mice of this strain.

The observation of type C murine leukemia virus particles in organs of the low-leukemia-strain mice with induced RCN and myeloproliferative lesions and in tissue cultures of spontaneous RCN derived from SJL/J strain mice and the serial transmission of induced RCN by cell-free filtrate strongly support viral etiology of this type of neoplasia.

INTRODUCTION

It is now well documented that spontaneous and induced neoplasms of the reticuloendothelial system occur in SJL/J strain mice (21, 29, 36, 40, 45, 48, 53) and in mice of other strains (2, 3, 20, 27, 30, 35, 42) as well as in hybrids (39, 41, 47, 51). Neoplasms of the reticuloendothelial system of mice include RCN types A and B, as described by Dunn (20). RCN type A has been described as a tumor of a single cell type, the reticulum cell (20). It has also been classified as a monocytic, histiocytic, or stem cell tumor (20, 21). Neoplasms in mice resembling Hodgkin's disease in man have been classified as RCN type B (20, 21, 36). Histologically, the RCN type B has been described as consisting of reticulum cells, lymphocytes, plasma cells, eosinophils, and neutrophils. Some of the neoplastic reticulum cells in mice resemble those of Hodgkin's disease in man (20, 37).

A high incidence (90%) of spontaneous RCS was reported by Murphy in mice of SJL/J strain at a mean age of 13 months (36, 37). Murphy described RCS type A and type B in these mice and considered type B to be histologically similar to Hodgkin's disease in man (36-39). The terms "reticulum cell sarcoma" and "reticulum cell neoplasm" have been used interchangeably as synonyms (20).

Investigators whose works relate to and suggest a viral etiology of RCN in mice include Gross (27), Moloney (35) and Stansley and Soule (49). Other investigators,

1 Presented at the 26th Annual Meeting of the Electron Microscope Society of America, New Orleans, La., September 16 to 19, 1968. This study was conducted under Contract PH43-65-604 (awarded to L.D.) within the Special Virus Cancer Program of the National Cancer Institute, NIH, USPHS.

2 Visiting Associate Professor of Virology, The University of Texas M. D. Anderson Hospital and Tumor Institute at Houston, and Visiting Associate Professor of Chronic Diseases, The University of Texas School of Public Health, Houston, Tex.

Received June 17, 1969; accepted August 11, 1969.

The abbreviations used are: RCN, reticulum cell neoplasms; RCS, reticulum cell sarcoma; HM, heterotopic myelopoiesis.
however, consider immunological disturbances as one of several factors responsible for RCS in mice (3, 31, 47, 50). The occurrence of RCS in mice following exposure to carcinogenic chemicals has also been reported (3, 50).

Yumoto and Dmochowski first reported the presence of virus particles morphologically identical with murine leukemia type C particles in organs of SJL/J strain mice with spontaneous RCN (53). They suggested a possible viral etiology of the neoplasms in mice of this strain. This report describes the induction and transmission of RCN and leukemias in mice of low-leukemia strains by extracts and cell-free filtrates of tumorous organs and extracts of tissue culture derived from neoplastic spleen of SJL/J strain mice with spontaneous RCN. A morphological description of the induced neoplasms at the microscopic and submicroscopic level is also presented.

MATERIALS AND METHODS

Animals

SJL/J strain mice with either RCN type A or mixed types A and B were used as source of material for preparation of extracts and for tissue culture studies. Embryos from an apparently normal SJL/J strain mouse were also used for tissue culture. SJL/J (CrT) strain mice (NCI Trust Stock—Cooperative Research—Texas) were kindly supplied by Mr. Samuel M. Poiley, Head of Mammalian Genetics and Animal Production Section, Cancer Chemotherapy National Service Center, National Cancer Institute, NIH, Bethesda, Md. Low-leukemia-strain mice were used for serial transmission experiments and as controls were BALB/c/KiDm, C3HeB/FeJ, C3Hf/BiDm, obtained from our own colony, and BALB/c/Dm/TEX, from Texas Inbred Mice Company (Houston, Tex.)

Preparation of Cell Extracts

Tissues of SJL/J strain mice with RCN and tissue culture derived from some of the following organs were used for preparation of the extracts.

Spleen and Liver of a 14-month-old SJL/J Strain Female Mouse with RCN Type A. The tissues were minced, ground with sterile sand in a cold mortar (4°), and suspended in Eagle’s solution as a 20% (w/v) extract. The suspension was centrifuged in the cold at 1,000 X g for 30 min. The supernatant was centrifuged at 7,000 X g for 10 min. The supernatant was removed and centrifuged at 93,000 X g for 90 min in the No. 40 rotor in the Spinco Model L2 ultracentrifuge. The high-speed centrifugal pellet was resuspended in 8 ml Eagle’s solution and the concentrated extract was used for inoculation.

Preparation of Filtrate

Spleen, lymph nodes, and thymus gland from a BALB/c strain mouse with induced RCN type A in the 5th serial transmission experiment were used for preparation of a 20% extract which was then centrifuged as described above. The resulting supernatant was filtered through a 0.45-μm membrane filter (Nalgene Filter Unit, NALGE, Sybron Corporation, Rochester, N. Y.). The filtrate was used for the 6th and 7th serial transmission experiments (Table 1). The filtrate in 0.2-ml quantities was injected i.p. into 2- to 7-day-old test mice.

Preparation of Material for Transplantation

A retroperitoneal tumor of RCN type A induced in a BALB/c strain mouse during the 4th serial transmission experiment (Table 1) was used for transplantation. The tumor was finely minced in a sterile Petri dish at room temperature and transplanted subcutaneously into four 1-month-old BALB/c strain mice by an 18-gauge trocar. This same technique was used to prepare subsequent transplants.

Tissue Culture

Spleen from an SJL/J strain mouse with RCN type A and, as control, embryos from an apparently normal
SJL/J Strain Retícula m Cell Neoplasms

Table 1

Serial transmission of RCN in low-leukemia-strain mice originally induced by an extract of spontaneous RCN of an SJL/J strain mouse

Figures in parentheses are for the low-leukemia C3H/f and C3HeB/FeJ strains. All other figures are for BALB/c strain mice. 6a, 7a, tumor transmission by cell-free filtrate of RCN induced in preceding passage. 6b, 7b, tumor transmission by supernatant of centrifuged RCN extract of preceding passage.

<table>
<thead>
<tr>
<th>Passage no.</th>
<th>No. of mice</th>
<th>Types of response of RCN</th>
<th>Average latent period (days)</th>
<th>Average life span of tumor-free mice (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SJL/J to BALB/c</td>
<td>With RCN/alive</td>
<td>A B AB HM* A or B and HM*</td>
<td>A B AB HM* A or B and HM*</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1/12</td>
<td>1 1 1 1</td>
<td>216 152 255 150 212</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3/11</td>
<td>1 1 4 4 1 3 (168) 192 123 113 119</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(9)/(14)</td>
<td>13/17 5 8 2 2 (10)/(15) (6) (2) (2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>23/33</td>
<td>10 1 5 5 6</td>
<td>135 139 139 112</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>18/36</td>
<td>3 1 8 8 6</td>
<td>95 172 140 157 124 119</td>
<td></td>
</tr>
<tr>
<td>6a</td>
<td>20/45</td>
<td>14 1 1 1 4</td>
<td>91 63 80 96 115</td>
<td></td>
</tr>
<tr>
<td>6b</td>
<td>12/16</td>
<td>1 1 4 4 7 91 98 91 132</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7a</td>
<td>4/14</td>
<td>1 1 2</td>
<td>44 44 44</td>
<td></td>
</tr>
<tr>
<td>7b</td>
<td>2/3</td>
<td>1 1</td>
<td>69 128 147</td>
<td></td>
</tr>
</tbody>
</table>

* HM with the histological characteristics of extramedullary proliferation of megakaryocytes and cells of the myeloid and erythroid series in lymph nodes, spleen, liver, and kidneys or lungs.

A number of inoculated animals are still alive.

SJL/J strain mouse were used for tissue culture. The tissues were minced and treated with 0.25% trypsin at an approximate ratio of 1 g tissue to 25 ml trypsin for 30 min at room temperature. The suspensions were centrifuged at 1000 X g for 10 min and the resulting pellets were washed and resuspended in Eagle's medium with 20% calf serum. The suspensions were put in T flasks and maintained at 37°.

Light and Electron Microscopy

Lymph nodes, spleen, thymus gland, liver, kidneys, lungs, and bone marrow were removed from tumorous mice for light and electron microscopy. The tissues for light microscopy were fixed in buffered formalin and stained with hematoxylin and eosin. Tissues selected for electron microscopy were cut into small pieces, fixed in 3% glutaraldehyde in Millonig's buffer (32) for 1 to 12 hr, postfixed with osmium tetroxide in Millonig's buffer (32) for 2 to 3 hr (46), dehydrated in graded ethanol solutions, treated with propylene oxide, and then embedded in Epon-Araldite mixture (33). The tissue cultures were scraped with a rubber policeman from the T flasks and centrifuged in tissue culture medium at 600 X g for 15 min. The supernatant was then removed and the pellet was fixed for 20 min in 3% glutaraldehyde (46). The remainder of the procedure for embedding was identical with that for solid tissues. Sections were cut with glass or diamond knives on a Porter-Blum MT-2 Sorvall Ultramicrotome, stained with a saturated 50% ethanol solution of uranyl acetate for 10 min, and counterstained with a solution of lead citrate for 10 min (44). Sections were examined in a Siemens Elmiskop 1A at magnifications varying from 1,500 to 20,000 diameters. The resulting electron micrographs were enlarged photographically as required.

RESULTS

Bioassays

Inoculation of the centrifuged extract (20%) of spontaneous RCN type A from a 14-month-old SJL/J strain mouse into BALB/c strain mice induced RCN type A in 1 of 12 inoculated animals after a latent period of 216 days (Table 1). Two of the outstanding lesions observed on serial transmission of the induced neoplasm were RCN and HM. The latter included proliferation of cells of the myeloid and erythroid series and of megakaryocytes. As shown in Table 1, the incidence of RCN and/or HM generally increased, while the average latent period of tumor appearance, particularly in development of RCN, generally decreased with consecutive serial transmissions. Control untreated animals of similar age and sex were free of these lesions.

The results of the inoculation of cell-free filtrates are also shown in Table 1. The results of transmission by supernatants of centrifuged homogenates of tumor tissues are comparable to those obtained by cell-free filtrates of the tumors.

The inoculation of a concentrated extract of spontaneous RCN from 4 SJL/J into 12 BALB/c strain mice induced RCN in 2 of the 12 inoculated animals (Table 2). Supernatant of centrifuged 20% extract of the RCN in-
Table 2
Serial transmission of RCN in BALB/c strain mice originally induced by a concentrated cell extract of spontaneous RCN of SJL/J strain mice

<table>
<thead>
<tr>
<th>Passage no.</th>
<th>No. of mice</th>
<th>Types of response of RCN</th>
<th>Average latent period (days)</th>
<th>Average life span of tumor-free mice (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SJL/J to BALB/c</td>
<td>With RCN/alive at time of 1st RCN</td>
<td>A</td>
<td>B</td>
<td>AB</td>
</tr>
<tr>
<td>1st</td>
<td>2/12</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2nd</td>
<td>1/3</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>3rd</td>
<td>9/10</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

* Heterotopic myelopoiesis with the histological characteristics of extramedullary proliferation of megakaryocytes and cells of the myeloid and erythroid series in lymph nodes, spleen, liver, and kidneys or lungs.

Table 3
Serial transmission of RCN in BALB/c strain mice originally induced by an extract from spleen tissue culture (passage 39) of an SJL/J strain mouse with spontaneous RCN

<table>
<thead>
<tr>
<th>Passage no.</th>
<th>No. of mice</th>
<th>Types of response of RCN</th>
<th>Average latent period (days)</th>
<th>Average life span of tumor-free mice (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SJL/J to BALB/c</td>
<td>With RCN/alive at time of 1st RCN</td>
<td>A</td>
<td>B</td>
<td>AB</td>
</tr>
<tr>
<td>1st</td>
<td>1/10</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2nd</td>
<td>16/23</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>3rd</td>
<td>10/16</td>
<td>7</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

* Heterotopic myelopoiesis with the histological characteristics of extramedullary proliferation of megakaryocytes and cells of the myeloid and erythroid series in lymph nodes, spleen, liver, and kidneys or lungs.

Transplantation Experiment

Minced retroperitoneal tumor (RCN type A) induced in a BALB/c strain mouse and implanted subcutaneously into four 1-month-old BALB/c mice grew in all 4 mice at the site of transplantation after a latent period of 1 week. In the second serial cell transplant, tumors developed after 7 days at the site of inoculation in all of 4 other inoculated 1-month-old BALB/c strain mice. All mice had enlarged inguinal, axillary, and mesenteric lymph nodes and enlargement of spleen. In the third serial transplant, all of 3 inoculated 1-month-old BALB/c strain mice developed tumors at the site of transplantation after a latent period of 1 week. They too showed enlargement of inguinal, axillary, and mesenteric lymph nodes and slight enlargement of spleen.

Gross Observations and Light Microscopy

Gross morphological changes in mice with induced RCN at autopsy varied. In most cases, the spleen, the lymph nodes, particularly mesenteric lymph nodes, Peyer’s patches of intestine, and the liver were enlarged. The kidneys, thymus gland, and lungs infrequently showed gross changes. There was no correlation between organ enlargement and the type of RCN induced.

Grossly abnormal tissues from a total of 162 mice comprising 94 (58%) males and 68 (42%) females were examined by light microscopy. The tissues included lymph nodes, spleen, thymus, liver, kidneys, lungs, and retroperitoneal or subcutaneous tumors.

RCN Type A. This type of RCN was mainly observed in liver (Fig. 1), mesenteric and peripheral lymph nodes, spleen, and thymus. Kidney involvement was rare.
several cases, typical RCN type A were present as retroperitoneal tumors which invaded skeletal muscle and the spinal column. The monotypic pleomorphic cells in these tumors were also observed in the mesenteric lymph nodes, spleen, liver, and the thymus gland of these animals. In the liver, diffuse sinusoidal and periportal infiltration of tumor cells was observed (Fig. 1) as described in SJL/J strain mice (53). RCN type A alone, or in combination with type B or HM, was present in a total of 106 (65%) animals of the 162 mice examined. There was no significant difference in distribution of the lesions according to sex.

**RCN Type B.** This type of RCN involving mesenteric lymph nodes, spleen, liver and thymus gland (Fig. 2) was seen infrequently as the sole tumor pattern (Tables 1 to 3). It was usually accompanied by the type A neoplasm in another part of the same organ or in another organ of the same animal. It was also found in animals with HM. The lesion was composed of large pleomorphic reticulum cells intermingled with lymphocytes, plasma cells, and eosinophilic myelocytes. Heterotopic megakaryocytes were not uncommon, either alone or in association with the type B neoplasm, in the liver (Figs. 3 and 4) and lymph nodes. Frequently, megakaryocytosis was observed in the spleen. As shown in Tables 1 to 3, the RCN type B or Hodgkin-like lesion was observed in 4 animals as the sole neoplasm and in 13 of the 162 animals in combination with RCN type A. The presence of both type A and type B RCN associated with HM was frequently observed (Tables 1 to 3).

All animals showing marked extramedullary hemopoiesis, including marked proliferation of erythroid, myelocytic, or megakaryocytic cells, are listed under HM. Some of these changes are illustrated in Figs. 3 to 5. On histological examination, transplants of induced RCN type A retained their original morphology. The enlarged lymph nodes, spleen, and liver of mice with transplanted RCN type A also showed tumor involvement, diagnosed as RCN type A.

Hyalinized degenerative changes in the glomeruli similar to those reported previously (41, 54) have been observed in the present study as a late development in some mice with RCN type A (Fig. 6).

**Electron Microscopy**

The cells shown in Fig. 7 are characteristic of RCN type A. The reticulum cells have large nuclei with large nucleoli and chromatin generally at the peripheral portion of the nucleus. In the cytoplasm, moderate numbers of mitochondria, granular endoplasmic reticulum, numerous free ribosomes, lipid droplets, and dense bodies are present. Golgi areas are fairly well developed. The appearance of cells in RCN type B is shown in Fig. 8. The reticulum cells are present among lymphocytes, eosinophils, plasma cells, and occasional neutrophils. The intercellular spaces are wider and more prominent than in RCN type A. The apparent difference in size and structure of mitochondria seen in the reticulum cells (Figs. 7 and 8) was not always observed.

Particles morphologically identical with type C murine leukemia virus were found in all organs examined of mice with induced RCN, HM, and combinations of the two. The number of virus particles observed in each organ varied. In general, virus particles were more numerous in lymph nodes and spleen than in other organs. No relationship could be established between the number of virus particles and the histological type of RCN observed.

Morphologically, 2 types of virus particles were seen. One type (so-called mature type C) was approximately 1000 Å in diameter with a nucleoid composed of an electron-dense fine reticular structure surrounded by an outer double membrane (Fig. 9). The other (so-called immature type C particle) was also frequently observed (Figs. 10, 13, and 16). Budding of virus particles was observed at the plasma membrane of reticulum cells and of apparently normal parenchymal cells (Figs. 9a, 11, 14a, and 15a). Several morphological variants of virus particles were found—among them, dumbbell particles (Fig. 10), cylindrical forms 0.2 to 2.0 μ long, chainlet forms (Fig. 13), and particles with tails (Fig. 14).

Both mature and immature type C virus particles were seen in the lymph nodes, spleen, thymus gland, liver, bone marrow, kidneys, and lungs. In the spleen and lymph nodes virus particles were present in the intercellular spaces and in cytoplasmic vacuoles of reticulum cells, lymphocytes, plasma cells, and macrophages (Fig. 10). Budding virus particles were observed at the plasma membranes of all these cells and of splenic eosinophils and neutrophils (Fig. 11). Numerous particles, including cylindrical forms, were observed in cytoplasmic channels of megakaryocytes in the spleen (Figs. 12 and 13). A similar distribution of mature, immature, and budding virus particles was observed in the thymus gland and in pulmonary alveolar capillaries, intercellular spaces, and pulmonary macrophages. In the liver, virus particles were found in the intercellular spaces, the lumen of sinusoids, and Disse's space (Fig. 15). Virus particles were also in the cytoplasmatic vacuoles of reticulum, Kupffer, and parenchymal cells. Budding virus particles were also observed at the plasma membrane of endothelial and parenchymal cells of the liver and infiltrating reticulum cells (Fig. 15a). Mature, immature, and budding type C virus particles were seen in the bone marrow. In kidneys, virus particles were in the lumen of glomerular capillaries, in extraglomerular capillaries, in hyalinized deposits within the glomerular tufts (Fig. 14), and in the cytoplasmatic vacuoles of epithelial cells of the proximal convoluted tubules. Budding virus particles were observed at the plasma membrane of capillary endothelial cells (Fig. 14a), epithelial cells of proximal convoluted tubules, and infiltrating reticulum cells.

Examination of spleen tissue cultures (19th, 39th, 99th, and 146th passages) derived from an SJL/J strain mouse with RCN type A showed comparatively small numbers of type C virus particles in cells of the 39th passage...
which was used for the transmission experiments. Large numbers of mature, immature, and budding type C virus particles were found in the tissue culture in the 99th and 146th passages (Fig. 16). In the embryo tissue culture from an apparently normal SJL/J strain mouse, type C virus particles were also observed (Fig. 17).

Another type of virus particle, approximately 800 Å in diameter, was frequently found in reticulum cells (Fig. 18), plasma cells, and lymphocytes. These virus particles have 2 concentric double membranes and are identical morphologically with intracisternal type A virus particles (6).

**DISCUSSION**

The histological appearance of the RCN types A and B, induced by supernatants of centrifuged cell extracts and by cell-free filtrates, was identical with that of the spontaneous RCN in SJL/J strain mice described by Yumoto and Dmochowski (53). RCN type A (62 out of 162 mice) was more prevalent than RCN type B alone (4 out of 162 mice) or RCN mixed types A and B (13 out of 162 mice). The prevalence of HM (46 out of 162 mice) approximates that of RCN type A as the second most common group of lesions in these animals. It is followed by the group of lesions with RCN type A or type B and HM (39 out of 162 mice). The incidence of the various histological types of the induced tumors in low-leukemia-strain mice was therefore different from that of spontaneous RCN in SJL/J strain mice previously reported (36–39, 53).

In the initial transmission experiments of spontaneous RCN from SJL/J to BALB/c strain mice, the RCN developed in 4 out of 34 inoculated animals after a latent period varying from 159 to 343 days (Tables 1 to 3). In comparison, the incidence of spontaneous RCN in control BALB/c strain mice of the sublines used in experiments was 1.9% in more than 300 animals 18 months of age or older. The incidence of spontaneous leukemia in BALB/c strain mice of the 2 sublines during 17 generations of inbreeding was 1% in mice 12 months of age or older. Examination of milk from control breeding mice of these sublines failed to reveal type C virus particles (16). Organs of these mice sampled at random were also negative for the presence of type C virus particles (unpublished data). This does not exclude the possible presence of small numbers of type C virus particles in these mice, in view of the known error of sampling by electron microscopy. It does, however, indicate the considerable number of virus particles in the tissues of tumorous mice.

The development of RCN in the inoculated BALB/c mice, rare as spontaneous lesions even at an advanced age, makes it apparent that the lesions were induced by the inoculum. The results of these experiments resemble those reported by Gross (24) in transmission of spontaneous leukemia from Ak high-leukemia-strain mice to C3H low-leukemia-strain mice. Gross (25–28) reported the potency of leukemia virus of mice with high incidence of spontaneous leukemia to be generally low when transmitted to low-leukemia-strain mice and that it increased considerably following successive serial passages through newborn hosts. The potency of Moloney leukemia virus increased after 8 serial passages in mice (35). In the present study, the incidence of RCN in BALB/c strain mice in the original passage also was low, and in subsequent transmission increased, while the average latent period decreased. The number of animals is too small for any conclusion to be drawn about the difference between the incidence of RCN in BALB/c strain mice inoculated with supernatants of 20% extracts of induced RCN and that of RCN in BALB/c strain mice inoculated with cell-free filtrate of induced RCN (Table 1).

Histological examination of RCN’s which served as the original source of material and of those induced in the serial transmission experiments demonstrated that the type of RCN induced could not be correlated with that which served as a source for transmission. Thus, transmission of RCN type A led to the induction of some or all of the types of tumors listed in Tables 1 to 3. As an overall observation, RCN type A was predominant in animals less than 1 year old, irrespective of the histological type of tumor which served as a source for inoculum. The occurrence of both types A and B in the same animal, sometimes even in the same organ, shows that a clear-cut distinction between the 2 types may be difficult. RCN of the types described including myeloproliferative disorders (HM), which histologically could be described as myeloid and erythroid leukemias, were the only neoplasms induced in the present study.

All examined tissues of mice with induced RCN and HM contained virus particles morphologically identical with murine leukemia type C virus (5). Similar particles have been observed in spontaneous RCN of SJL/J strain mice (53). No difference in the morphological appearance could be observed between the virus particles seen in the present study and those reported in mice with leukemia spontaneous or induced by Passage A, Moloney, Friend, and Rauscher viruses (4, 6, 7–13, 15, 16, 17, 22, 27, 28, 34, 52, 54, 55). However, none of the animals in the present study developed lymphoid leukemia. The morphological variants of virus particles found in the present study, i.e., dumbbell, cylindrical, and chainlet forms, etc., have also been demonstrated in tissues and in tissue cultures of leukemic organs from mice or rats with induced leukemia (4, 7, 12, 28, 34, 43, 53, 55). Intracisternal type A virus particles (5) as found in the present study have also been reported in SJL/J strain mice with spontaneous RCN (53). The significance of these virus particles remains to be resolved.

Numerous type C virus particles and budding particles found even in the 146th passage of the tissue culture of tumorous spleen from an SJL/J strain mouse with spontaneous RCN indicate that the virus multiples for prolonged periods of time in tissue culture. The tumor-inducing activity of the extract of cells from the 39th passage of the spleen tissue culture (Table 3) con-
firms the morphological finding of virus particles in this culture and their biological potency.

Repeated light microscopic examination of supernatants obtained by centrifugation of extracts of cell homogenates at 7000 × g for 10 min preceded by centrifugation at 10000 × g for 30 min failed to reveal the presence of cells in these supernatants except for the presence of cellular debris. Since these lesions were induced by cell-free filtrates as well as by supernatants of centrifuged tissue extracts, the type C virus particles may be regarded as the causative agent. The observation of murine leukemia type C particles in all RCN, irrespective of the histological type, indicates that individual host factors play an important part in the development of the various types of RCN even in inbred mice of the same origin and the same age. However, the possibility of morphologically similar particles having different biological activity must not be overlooked.

No difficulty was encountered in transplantation of type A RCN in BALB/c strain mice. Although a comparison of the behavior of induced RCN type A and type B on transplantation in BALB/c strain mice would have been desirable, that aspect was outside the scope of the present study.

It is now well known that vertical transmission of leukemia virus occurs in mice (23, 24). In the present study, type C virus particles found in the embryo tissue culture from an apparently normal SJL/J strain mouse suggest that vertical transmission of the virus occurs through embryos in the SJL/J strain.

The induction and serial transmission of RCN, including type B or "Hodgkin's-like lesions" in BALB/c strain mice and in mice of other strains, may have some bearing on the etiology of reticulum cell sarcoma and Hodgkin's disease in man. The possibility that a virus or viruses may be implicated in the etiology of Hodgkin's disease has been suggested (1, 13-15, 18, 19). Occasional particles resembling murine leukemia type C virus particles were found in lymph nodes of patients with Hodgkin's disease (19). The results of the present study lend support to further search for a virus as an etiological agent in RCS and Hodgkin's disease of man.

ADDENDUM

In an experiment now in progress, six 1- to 3-day-old SJL/J strain mice received an i.p. inoculation of a cell-free filtrate prepared from the spleen, thymus, and lymph nodes of a BALB/c strain mouse with induced RCN type A (7th passage, Table I). To date, 2 of the 6 inoculated mice were killed when they appeared to be sick, at the ages of 8 and 9 weeks. On postmortem examination of each animal, the peripher- al and visceral lymph nodes were greatly enlarged; the liver and spleen were also enlarged. The kidneys showed diffuse areas of infiltration. On histological examination, all these organs were replaced or infiltrated by RCN type A cells. Examination of ultrathin sections of one of the lymph nodes disclosed large numbers of mature and immature type C virus particles in the intercellular spaces and budding from the plasma membranes. Comparable histological changes were absent in organs of control SJL/J animals of the same age and sex. The RCN type A observed in the inoculated SJL/J strain mice is considered to be induced by a virus derived from the tumor-bearing BALB/c strain mouse (7th passage). Further evidence of induction and/or acceleration of the RCN in the 2 SJL/J mice is based on the fact that the tumors developed within 2 months, while the incidence of "spontaneous" RCN in SJL/J strain mice of the colony from which the mice were derived is 16.6% at an average age of 320 days (53). At the conclusion of the experiment—during the printing of this paper—5 of the 6 inoculated mice developed RCN at the following ages: 56, 64, 77, 77, and 92 days.

REFERENCES


MARCH 1970

735

Downloaded from cancerres.aacrjournals.org on April 20, 2017. © 1970 American Association for Cancer Research.
Fujinaga, Poel, Williams, and Dmochowski


47. Schwarz, R. S., and Beldotti, L. Malignant Lymphomas following Allogenic Disease: Transition from an Immunological to a Neoplastic Disorder. Science, 149: 1511-1514, 1965.


Downloaded from cancerres.aacrjournals.org on April 20, 2017. © 1970 American Association for Cancer Research.
Fig. 1. Liver with RCN type A, showing proliferating cells in the periportal area, involvement of the hepatic sinusoids, and a megakaryocyte in the periportal vein. × 560.

Fig. 2. RCN type B. Neoplastic reticular cells in spleen replace white pulp of the peritrabecular region (right). × 520.

Fig. 3. Liver with histological pattern of myeloid leukemia and occasional heterotopic megakaryocyte. Neoplastic cells coat the surface of the liver and distend the sinusoids. × 520.

Fig. 4. Megakaryocytosis in liver of a mouse. × 560.

Fig. 5. RCN type A with features of erythroid leukemia. Stem cells, erythroblasts, and distended liver sinusoids compress the hepatic cords. × 560.

Fig. 6. Hyalinized capillary tufts in renal glomerulus of a mouse with RCN type A. Same case as shown in Fig. 1. Monotypic, neoplastic reticular cells surround Bowman's capsule, the urinary pole, and collecting tubules. × 850.

Fig. 7. Low-power electron micrograph of mesenteric lymph node of a BALB/c strain mouse with RCN types A and B in the third serial passage. Monotypic cells in this field characterize RCN type A. Reticulum cells have large nuclei with prominent nucleoli. In the cytoplasm, a moderate number of mitochondria, granular endoplasmic reticulum of tubular form, and large numbers of ribosomes may be seen. × 5,500.

Fig. 8. Low-power electron micrograph of a mesenteric lymph node of a BALB/c strain mouse with RCN type B in the original passage. Reticulum cells (R), lymphocytes (L), an eosinophil (E), and neutrophils (N) are present. × 5,000.

Fig. 9. Part of the spleen from a BALB/c mouse with RCN types A and B induced by original SJL/J inoculum. Mature type C virus particles are in the intercellular space. Arrows, double outer membranes. × 80,000.

Fig. 9a. Two budding virus particles at the plasma membrane of a reticulum cell infiltrating the lung of a BALB/c strain mouse with RCN type A in the third serial passage. × 80,000.

Fig. 10. Part of a macrophage in mesenteric lymph node of a BALB/c strain mouse with heterotopic myeloid and erythroid proliferation in the third serial passage. Mature and immature virus particles (arrow) may be seen in cytoplasmic vacuole of the cell. Dumbbell forms of virus particles with structure of immature virus may be seen (double arrows). × 80,000.

Fig. 11. Part of a reticulum cell in mesenteric lymph node of BALB/c strain mouse with HM in the first serial passage. Budding virus particles may be seen at the plasma membrane of the cell (arrows). × 60,000.

Fig. 12. Low-power electron micrograph of a megakaryocyte in the spleen of a BALB/c strain mouse with RCN type A in the fourth serial passage. Numerous virus particles may be seen in the cytoplasmic channels. × 5,500.

Fig. 13. Part of a megakaryocyte in the spleen of a BALB/c strain mouse with RCN type B and heterotopic myeloid proliferation in the third serial passage. Numerous immature virus particles are present (single arrow). One cylindrical form with the inner structure of immature virus particles is located in the center. Some of the particles are connected and form chainlet figures (double arrows). × 60,000.

Fig. 14. Hyalinized deposit within glomerular capillary in the kidney of a BALB/c strain mouse with RCN type A and HM in the fourth serial passage. Numerous mature virus particles may be seen in the amorphous substance in the lumen of the capillary. Some "hand-mirror" forms of virus particles (with tails) may be seen (arrow). × 27,500.

Fig. 14a. Budding virus particle (arrow) at the plasma membrane of endothelial cell of glomerular capillary in the kidney of a BALB/c strain mouse with RCN type A in the fifth serial passage. × 60,000.

Fig. 15. Liver of a BALB/c strain mouse with RCN type A and heterotopic myeloid proliferation in the third serial passage. Virus particles may be seen in Disse's space (arrow). × 21,500.

Fig. 15a. Budding virus particle at the plasma membrane of a parenchymal cell of liver of a BALB/c strain mouse with RCN type A and heterotopic myeloid proliferation in the second serial passage. × 60,000.

Fig. 16. Immature virus particles (arrow) in 146th serial passage of tissue culture of tumorous spleen from an SJL/J strain mouse with spontaneous RCN type A. One particle appears to be a budding virus (double arrow). × 60,000.

Fig. 17. Mature virus particles in embryo tissue culture derived from an apparently normal SJL/J strain mouse. × 60,000.

Fig. 18. Part of a reticulum cell in the mesenteric lymph node of a BALB/c strain mouse with RCN type A in the first serial passage. Intracisternal type A virus particles (arrows) are present in the lumen of what appears to be endoplasmic reticulum of the cell. × 60,000.
Biological and Morphological Studies of SJL/J Strain Reticulum Cell Neoplasms Induced and Transmitted Serially in Low-Leukemia-Strain Mice

Susumu Fujinaga, William E. Poel, W. Clydell Williams, et al.


Updated version

Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/30/3/729

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.