Avian myeloblastosis virus-induced lymphosarcoma producing erythroblastic leucosis in chicks

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Abstract

Acute myeloblastosis and several forms of tumor, including one case of lymphosarcoma occurred when avian myeloblastosis virus (BAI-A strain) was inoculated into newly hatched chicks (SPF). The homogenate of lymphosarcoma inoculated intraperitoneally into other newly hatched chicks induced a high incidence of erythroblastic leucosis. Electron microscopy did not reveal the presence of C-type virus particles in the tumor tissue. The relationship between avian myeloblastosis virus, lymphosarcoma and erythroblastic leucosis is discussed.

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AVIAN MYELOBLASTOSIS VIRUS-INDUCED LYMPHOSARCOMA PRODUCING ERYTHROBLASTIC LEUCOSIS IN CHICKS

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Abstract: Acute myeloblastosis and several forms of tumor, including one case of lymphosarcoma occurred when avian myeloblastosis virus (BAI-A strain) was inoculated into newly hatched chicks (SPF). The homogenate of lymphosarcoma inoculated intraperitoneally into other newly hatched chicks induced a high incidence of erythroblastic leucosis. Electron microscopy did not reveal the presence of C-type virus particles in the tumor tissue. The relationship between avian myeloblastosis virus, lymphosarcoma and erythroblastic leucosis is discussed.

The BAI-A strain of avian myeloblastosis virus (AMV) induces not only acute myeloblastosis but also visceral lymphomatosis, nephroblastoma, and osteopetrosis (1). At present, little is known about the oncogenic mechanism. According to Burmester et al. (2) inoculation with a high concentration of this virus induces erythroblastosis and inoculation with a lower concentration induces lymphomatosis. It is uncertain whether the manifested form of leucosis is dependent upon virus concentration or upon contaminated clonal virus specific for the disease (1, 2). To examine the factors determining the form of leucosis, we used AMV (BAI-A strain) and induced various forms of leucosis in chicks (SPF). One case of lymphosarcoma was found in this sample. This lymphosarcoma differed from other reported leucoses because electron microscopy did not reveal the presence of C-type viral particles in the tumor tissue and the homogenate of the tumor induced acute erythroblastic leucosis at high incidence. In the present study, we report on this newly found tumor and discuss the mechanism of avian tumor induction by AMV.

MATERIALS AND METHODS

Virus inoculation: The BAI-A strain of AMV was obtained through the courtesy of Dr. T. Shimizu, National Institute of Animal Health, Japan Agri-

Abbreviations: SPF, specific pathogen free; AMV, avian myeloblastosis virus; PBS (−), phosphate buffer saline, divalent cation free.

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cultural Ministry at Tokyo, as frozen dried blood plasma from myeloblastosis chicks. This sample was resolved aseptically in minimum essential medium (MEM) and was inoculated intraperitoneally into chicks within 24 hours after hatching. Chicks attacked by acute myeloblastosis were exanquinated by heart puncture, and the blood plasma collected. This plasma was diluted with PBS (−), and the diluted plasma was injected intraperitoneally into the next series of chicks within 24 hours after hatching.

**Chicks:** White leghorn (SPF) chicks were kindly supplied by Dr. T. Kan, Research Institute of Microbiology, Osaka University, Kannonzi. They were free of leucosis virus in immunological investigations at the same institute (3). Leucosis or Marek's disease did not occur during the 150-day observation period in the 30 chicks of the control group.

**Diagnosis of various forms of the disease:** Onset of myeloblastosis was checked by hematocrit examination, and the peripheral blood picture was investigated microscopically after Giemsa staining. Tumors were examined at autopsy. Lymphosarcoma was diagnosed after staining by hematoxylin-eosin and silver impregnation, and observed by electron microscopy.

**Maintenance and observation of chicks:** The inoculated groups and the control group of chicks were kept in separate cages in separate rooms. All cages were sterilized before use. Food and water were separated between the two groups and never interchanged. The manifestation of leucosis was examined on blood smears and by hematocrit readings semi-weekly from 3 days after inoculation in all birds. ATPase activity of myeloblastic chick plasma was measured by the method of Beaudreau and Becker (4). It was reported that AMV containing plasma showed high ATPase activity (5-7). Postmortem examination was performed on all birds. Various forms of leucosis occurred in the experimental groups. A leg tumor was found at 70 days after inoculation (Figs. 1 and 2). Postmortem examination was performed on this tumor-bearing chick. The tumor, liver, kidney, and bone marrow were fixed with 10% formalin and stained with hematoxylin-eosin and silver impregnation method for microscopic examination, as described previously. Electron microscopic examination was performed on the tumor. The tumor was stored in a deep freezer at −80°C, thawed at room temperature, and homogenized vigorously up and down ten times in PBS (−) with a teflon homogenizer. The homogenate was injected intraperitoneally into chicks, and the chicks were observed for 90 days and diagnosed, as described previously.

**Electron microscopy:** The HU-11C electron microscope was used for observations of virus particles in myeloblastic chick bone marrow, lymphosarcoma and other tumors. The samples were fixed in 2.5% glutaraldehyde and 1% osmium tetroxide in 0.1M cacodylate buffer, pH 7.2 and dehydrated in series with ethyl alcohol and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate.
RESULTS

In vivo infectivity of AMV

The intraperitoneal injection of AMV in newly hatched chicks induced acute myeloblastosis, nephroblastoma, ovarian tumor and lymphosarcoma. Injection of the myeloblastosis chick plasma induced myeloblastosis and nephroblastoma in the next experimental series (Table 1).

| Table 1. Induction of various forms of neoplasma by BAI-A strain of AMV |
|-----------------------------|-----------------|-----------------|-----------------|-----------------|
| Series          | Chicks (No.) | Myeloblastosis | Nephroblastoma | Other tumor |
| 1st            | 11            | 4              | 3              | 2              |
| 2nd            | 15            | 4              | 3              | 0              |
| 3rd            | 23            | 3              | 2              | 0              |

Myeloblastosis chick plasma was diluted with PBS (-), and a 0.5 ml aliquot was injected to the next series of chicks (150 days observation).

Leg tumor (lymphosarcoma)

Microscopic examination after hematoxylin-eosin staining and silver impregnation method confirmed that the tumor was lymphosarcoma (Fig. 3). Giemsa-stained peripheral blood smears of the tumor-bearing chick showed a normal pattern, and the ATPase activity of the blood plasma was negligible. The liver, kidney, and bone marrow appeared normal both macro- and microscopically (Fig. 2). Electron microscopic examination of the lymphosarcoma showed degenerative changes in the cytoplasm. Endoplasmic reticulum and mitochondria were not distinctly observable. C-type particles of avian leucosis virus were not detected in the intra-cisternal and intercellular spaces (Fig. 4).

Myeloblastosis

This disease occurred between the second to fifth week after virus inoculation. The peripheral blood cells were predominantly immature white cells and mainly myeloblasts. Hematocrit values of the peripheral blood of attacked chicks were about 50% of the control values. A number of C-type virus particles of approximately 100 nm in diameter were observed in the bone marrow by electron microscopy (Fig. 5). Some cases were complicated with nephroblastoma. The ATPase activity of myeloblastosis plasma was higher than that of normal plasma.

Manifestation of newly induced erythroblastic leucosis

The homogenate of the lymphosarcoma induced erythroblastic leucosis at high incidence. The onset was 2–4 weeks after the inoculation of the homogenate, and the peripheral blood cells were mainly erythroblastic cells (Fig. 6). The hematocrit values of chicks with erythroblastic leucosis were
Fig. 1. Gross appearance of AMV induced lymphosarcoma in the chick.
Fig. 2. Gross appearance at autopsy of the lymphosarcoma-bearing chick. Part of the tumor was enclosed with hematoma and retroperitoneal organs were observed to be normal.
Fig. 3. Microphotograph of the lymphosarcoma stained by the silver impregnation method (×100).
Fig. 4. Electron micrograph of the lymphosarcoma cells. C-type virus particles were not observed in tumor cells (×9,000).
Fig. 5. Electron micrograph of a myeloblastosis chick bone marrow cell. C-type virus particles are observed in the cytoplasm (×20,000).
Fig. 6. Peripheral blood smear of chick erythroblastic leucosis induced by the lymphosarcoma homogenate. Erythroblastic cells are round in shape. The cytoplasm is polychromatic and the nuclei have a cartwheel-like appearance (×400).

Table 2. Induction of Erythroblastic Leucosis by the Lymphosarcoma Homogenate

<table>
<thead>
<tr>
<th>Series</th>
<th>Chicks [No.]</th>
<th>Erythroblastic leucosis [No.]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>2nd</td>
<td>89</td>
<td>33</td>
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about 50% of the normal values. Table 2 showed the frequency of this disease after homogenate inoculation. The ATPase activity in the leucosis plasma was negligible. These results were consistent with the results of Bonar et al. (8). During the three month observation period, no other types of leucoses were found in the tested chicks.

DISCUSSION

It was reported (11) that R strain, BAI-A strain, and RPL strain of avian leucosis virus induced erythroblastic leucosis, myeloblastosis, and visceral lymphomatosis, respectively. However, Baluda et al. (1, 9, 10) reported that clonal virus form BAI-A strain of AMV induced various forms of tumors in chicks and that mesenchymal cells were target cells for this virus. On the other hand, Burmester’s group (2) speculated that the induction of different types of tumor was dependent on the viral concentration used for inoculation. It is still unknown whether the virus strain is multipotent in tumor induction or whether various types of virus are mixed in the BAI-A strain. In the present study a solitary lymphosarcoma was induced in a chick (SPF) by BAI-A strain of AMV. Although C-type viruses were not detected in the lymphosarcoma by electron microscopy, the homogenate of the tumor induced erythroblastic leucosis in chicks. These results may suggest that BAI-A strain of AMV was either contaminated by another strain of virus or induced lymphosarcoma under usual conditions. These results also suggest that a small amount of virus (not observed by electron microscopy) was present or that oncogenic factors were produced in the lymphosarcoma cell by recombination or phenotypic mixing. The oncogenic factors may have induced erythroblastic leucosis. The results of the present study show that a clonal virus may be altered by passage through a living system. Further investigation is in progress on the relationship between AMV and this sarcoma.

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