Rapid Induction of Lymphoid Leukemia and Ascites by Avian Leukosis Virus from a Lymphoid Leukemia Cell Line

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ABSTRACT. To examine whether a lymphoid leukemia (LL) cell line releases an LL-specific avian leukemia virus (ALV) or not, two viral materials, culture fluid and a concentrated viral material from an LL-cell line, were inoculated into a total of 74 day-old chicks of line 15I in 5 experiments. Spectrum of diseases induced, their incidence and incubation periods to onset were examined. Fifteen chicks were inoculated with the culture fluid and 9 (60%) developed ascites [59–119 days post inoculation (dpi)]; geometric mean (GM) of dpi, GM: 89.6], but LL was not induced in any chicks inoculated. Fifty-nine chicks were inoculated with the concentrated viral material and LL was recognized in 13 (22.0%) (27–74 dpi; GM: 48.4), ascites with LL in 11 (18.6%) (34–75 dpi; GM: 41.3), ascites alone in 21 (35.6%) (32–83 dpi; GM: 48.2), erythroblastosis in 2 (3.4%) (70–102 dpi; GM: 84.5), and other diseases in 12 (20.3%) (43–102 dpi; GM: 61.8). LL lesions were frequently observed in the liver, spleen, kidneys, bursa of Fabricius (bursa), bone marrow and gonads. Mild lymphocytic foci in some visceral organs and perivascular cuffing in the central nervous system were observed mainly in several chicks diagnosed as having complication of ascites with LL or other diseases. In addition to these lesions, atrophy of bursa and thymuses was recognized in them. No antibodies against Marek's disease virus (MDV) and reticuloendotheliosis virus were detected in 36 sera taken from the chicks inoculated with the concentrated viral material. Serotype 2 MDV was isolated from the buffy coat of some inoculated chicks. These results suggest that the properties of ALV inoculated and immunosuppression caused by inoculation with high doses of ALV are involved in rapid induction of LL and expression of pathogenicity of serotype 2 MDV released from the LL cell line and included in the viral inoculum. This is the first report describing the rapid induction of LL and ascites in chicks. — KEY WORDS: ascites, avian leukemia virus, lymphoid leukemia, Marek's disease virus.

Avian lymphoid leukemia (LL) is B-cell lymphoma caused by an avian leukemia virus (ALV) infection. It originates in the bursa of Fabricius (bursa) and metastasizes to other visceral organs such as the liver, spleen, and kidneys after a long incubation period of several months or more [18, 19, 27]. It is generally believed that cancer is the final product of a multistep process presumably involving multiple genes. From this viewpoint, the initiation and progression of LL has been considered as a model case for the involvement of at least two different genes in carcinogenesis [3]. It has been suggested that the first, most likely c-myc [13], induces follicular hyperplasia [1, 26] and the second, c-bic [10], causes the formation of bursal tumors. That is, c-myc and c-bic act synergistically during lymphomagenesis and that c-bic is involved in later stages of tumor progression [10]. However, these data cannot explain the long incubation period to LL onset, and the mechanisms of LL lymphomagenesis are still unclear.

The best way to clarify the mechanisms of LL lymphomagenesis is considered to be the establishment of a system which rapidly induces LL and the analysis of the factors responsible for the progression of the neoplastic condition. However, nobody has yet been successful in creating these experimental conditions so far. We have already isolated tumor-specific viruses from ALV-induced tumors. Namely, avian erythroblastosis virus strain H (AEV-H) from erythroblastosis [20] and avian sarcoma viruses (S1 and S2 strains) from sarcomas [16], although their titters were very low compared to the laboratory strains when they were isolated.

Therefore, to examine whether an LL-cell line releases an LL-specific virus or not, two viral materials, culture fluid and a concentrated viral material from an LL-cell line, were inoculated into chicks of line 15I.

We report here the first example of LL and ascites rapidly induced by inoculation with a large amount of ALV and a small amount of serotype 2 Marek's disease virus (MDV), both released from an LL-cell line and included in the inoculum [14], and also discuss LL-lymphomagenesis, pathogenesis of ascites and interaction between ALV and serotype 2 MDV on the pathogenicity of the diseases induced.

MATERIALS AND METHODS

Chicks and chicken embryos: All the newly hatched chicks and chicken embryos used were derived from a specific-pathogen-free (SPF) flock of White Leghorn chickens, line 15I [7, 18]. Its leukemia-sarcoma virus susceptibility phenotype was C/E (resistant to infection with the virus of subgroup E) or C/CE. C/AE phenotype cells,
originally derived from a flock of line 15I [16], were recovered from a frozen state in liquid nitrogen. Chicken embryo fibroblast (CEF) cells were cultured in F10 medium containing 10% tryptose phosphate broth (TPB), 5% bovine serum, 0.5% heat-inactivated chicken serum, and antibiotics in a CO₂ incubator at 38°C as described previously [16].

Virus inoculated: Two viral materials were prepared from LSCC-BK3, clone A (BK3A) cells [17]. The cells were cultivated in F10 medium supplemented with 10% TPB, 5% fetal calf serum, 0.5% heat-inactivated chicken serum and antibiotics in a CO₂ incubator at 38°C as already described [17]. The culture fluid when the cells grew actively was collected by low-speed centrifugation, and the resulting supernatant, which contained 10⁷ tissue culture infective dose (TCID) per ml of subgroup A ALV, was used as a viral material following 3 cycles of freezing and thawing. Another viral material was prepared as follows: the supernatant was passed through a 450 nm membrane filter, and the filtrate was concentrated by a modification of the method described previously [4]. Briefly, polyethylene glycol (PEG) 6,000 (Wako Pure Chemical Industries, Ltd., Tokyo, Japan) was added to the filtrate at a concentration of 8 g/100 ml, and the mixture was stirred at 4°C overnight. The mixture was centrifuged at 6,000 rpm for 30 min, and the precipitate was resuspended in a Tris-buffered solution at pH 7.4 in 1/100 of the original volume, and stored at −80°C before use. The concentrated virus material contained 10⁸ TCID per ml of ALV subgroup A and 10 plaque-forming units (PFU) per ml of serotype 2 MDV when they were titrated as described below.

Titration of ALV: Titration of the viral inoculum for chicks was performed by the RIF test as described previously [16, 20, 30].

Titration of MDV in the concentrated viral material and identification of MDV serotype: During the histopathologic examination of various organs of chicks inoculated with the viral inoculum, Marek’s disease (MD)-like lesions such as lymphocytic infiltration in the central nervous system (CNS) and in the peripheral nerves were recognized in a few chicks in Exp. 1 through Exp. 3 as described in the Results. Reticuloendotheliosis virus (REV) infection induces peripheral nerve lesions similar to those of MD in chicks [34]. Therefore, we examined the presence of MDV and REV, both in the viral inoculum and in the peripheral blood lymphocyte (PBL) of chicks inoculated with the viral inoculum in Exp. 4, in addition to the examination of antibodies of the inoculated chicks to MDV or REV as described below.

Titration of MDV and identification of MDV serotype were carried out as follows. A ten-fold dilution was made from the concentrated viral material and 0.1 ml of each dilution was inoculated into C/AE CEF cultures, which had been transferred at least three times, at 3- or 4-day intervals. When round type cytopathic effect (CPE) appeared in the culture, the culture was fixed with cold acetone, and the serotype of MDV was determined by the indirect immunofluorescent antibody (IFA) test. Monoclonal antibodies to serotypes 1 and 2 MDV, and to serotype 3 herpesvirus of turkey (HVT) [23, 24, 36] were prepared by Dr. Kondo, Department of Epizootiology, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Japan. The fluorescent isothiocyanate (FITC)-conjugated rabbit anti-mouse immunoglobulin G (IgG) (Seikagaku Kogyo, Co., Tokyo, Japan) was purchased.

Examination of REV in the concentrated viral inoculum: To examine REV in the viral inoculum, the C/AE CEF cultures inoculated with 0.1 ml volumes of the undiluted material were transferred at least three times, at 3- or 4-day intervals and finally grown in wells of a Heavy Teflon Coating Slide (Bokusui Brown Co., Tokyo). After washing in phosphate buffered saline (PBS), the CEF cells were air-dried, fixed in cold acetone and dried again. The CEF cell antigens were incubated with anti-REV chicken serum, and then, FITC-conjugated rabbit anti-chicken Ig (Cappel Lab., PA, U.S.A.) for IFA tests as already described [21, 37].

Virus inoculation into chicks and housing: Chicks of less than 2 days old were given an intra-abdominal inoculation of 0.2 ml of each viral material. The chicks, throughout the experimental period, were sheltered in a house equipped with filtered air, and a negative-pressure ventilation system.

Assay for MDV and REV antibodies: Antibodies to MDV were examined by agar-gel precipitin test (AGP) tests [5, 37] and enzyme-linked immunosorbent assay (ELISA). The AGP and ELISA antigens of MDV were prepared from CEF infected with the 2H strain of serotype 2 MDV isolated from BK3A [14]. The AGP test was carried out as described previously [37]. An ELISA antigen of MDV was prepared by the methods of Cheng e al. [9]. The ELISA procedure was conducted by methods already described [33]. Antibodies to the T strain of REV [29] were examined by the AGP tests [34, 37].

Examination of MDV and REV in inoculated chicks: Chicken kidney (CK) cell cultures were obtained from 10-week-old line 15I chickens by methods already described [21, 22]. The PBL of chicks inoculated with the concentrated viral material was obtained from the blood samples using Lymphoprep (Nyegaard & Co. Asl. Oslo, Norway). The PBL suspension (0.2 ml containing 10⁷ cells) was inoculated into each of the duplicate monolayer cultures of 48 hr-old primary CK ce ls. Foci of CPE (plaques) were enumerated under a light microscope 7-10 days post inoculation (dpi) in CK cells, as described previously [32]. Identification of the MDV serotype isolated was carried out by the methods described above. To examine the infection of chicks with REV, the CEF antigen was prepared from C/AE CEF cultures inoculated with the PBL for IFA tests by the methods described above.

Diagnosis: A diagnosis was tentatively made on the basis of autopsy findings as follows. The chicks with nodular lesions in the bursa and visceral organs were diagnosed as having LL. The chicks with both ascites and nodular lesions in bursa and some visceral organs were diagnosed as having complication of ascites with LL. The chicks with ascites exclusively were diagnosed as having ascites.
Erythroblastosis was diagnosed by autopsy findings and hematological examination. The diagnosis of chicks with mild swelling of the liver without nodular lesions in the bursa, or chicks without any macroscopic lesions was made as others. After macroscopic observation, tissue specimens were collected from various organs such as the liver, spleen, kidneys, heart, lungs, proventriculus, duodenum, cecum tonsil, bursa, thymuses, gonads, femoral bone marrow, brain, spinal cord, and sciatic nerves. They were fixed in 10% buffered formalin, embedded in paraffin, cut into sections, and stained with hematoxylin and eosin (HE) for microscopic observations.

The nomenclature of the disease of the avian leukemia/ sarcoma group was based on the description of Payne and Purchase [27].

RESULTS

Incidence of the diseases induced by a viral material (culture fluid) and their incubation period: Of the 15 chicks inoculated, ascites was recognized in 9 chicks (60%) 59–119 dpi [geometric mean (GM) of dpi: 89.6 days] and erythroblastosis appeared in two chicks (13.3%) 59–159 dpi (96.8 days). Four chicks (26.7%) were classified as others. LL was not observed in any chicks (data not shown). Macroscopic and microscopic lesions of ascites were similar to those induced in chicks inoculated with the concentrated viral material described below.

Incidence of the diseases induced by the concentrated virus material and their incubation period: Table 1 summarizes the results obtained when the concentrated viral material was inoculated into chicks. The experiment was repeated 4 times and a total of 59 chicks were used in these experiments.

Table 1. Incidence of diseases and incubation period in chicks inoculated with a viral material prepared from an LL-cell line (LSCC-BK3A)

<table>
<thead>
<tr>
<th>Exp. No. of chicks</th>
<th>Diseases induced</th>
<th>Observation period (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LL(^a)</td>
<td>As + LL(^b)</td>
</tr>
<tr>
<td>1</td>
<td>12</td>
<td>36(^b)</td>
</tr>
<tr>
<td></td>
<td>60.0 (53–74) (^g)</td>
<td>75</td>
</tr>
<tr>
<td>2</td>
<td>17</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>37.5 (27–52)</td>
<td>39.2 (34–52)</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>44.9 (37–51)</td>
<td>45.8 (37–51)</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>52.7 (51–54)</td>
<td>41.6 (34–51)</td>
</tr>
<tr>
<td>Total</td>
<td>59</td>
<td>13 (22.0(^h))</td>
</tr>
</tbody>
</table>

\(^a\) Lymphoid leukemia. \(^b\) Complication of ascites (As) with LL. \(^c\) Erythroblastosis. \(^d\) Number of chicks affected. \(^e\) Of two cases, one was complicated with LL. \(^f\) Average incubation period in days (geometric mean). Number in parentheses shows the shortest and the longest days. \(^g\) Number of chicks affected. The percentage of the chicks affected to the number of chicks inoculated is shown in parentheses.
Table 2. Incidence of macroscopic and microscopic lesions in various organs of chicks diagnosed as having lymphoid leukemia (LL)

<table>
<thead>
<tr>
<th>Organs</th>
<th>Macroscopic lesion</th>
<th>LL lesion</th>
<th>Lymphocytic infiltration</th>
<th>Other lesion</th>
<th>No lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>13/13</td>
<td>7/7</td>
<td>0/7</td>
<td>0/7</td>
<td>0/7</td>
</tr>
<tr>
<td>Spleen</td>
<td>12/13</td>
<td>7/7</td>
<td>0/7</td>
<td>0/7</td>
<td>0/7</td>
</tr>
<tr>
<td>Kidney</td>
<td>8/13</td>
<td>6/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
</tr>
<tr>
<td>Heart</td>
<td>2/13</td>
<td>4/7</td>
<td>2/7</td>
<td>1/7</td>
<td>0/7</td>
</tr>
<tr>
<td>Lung</td>
<td>0/13</td>
<td>0/7</td>
<td>0/7</td>
<td>0/7</td>
<td>2/7</td>
</tr>
<tr>
<td>Bursa of Fabricius</td>
<td>13/13</td>
<td>7/7</td>
<td>0/7</td>
<td>0/7</td>
<td>0/7</td>
</tr>
<tr>
<td>Thymus</td>
<td>5/13</td>
<td>6/7</td>
<td>0/7</td>
<td>0/7</td>
<td>1/7</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>7/13</td>
<td>5/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>Proventriculus</td>
<td>1/13</td>
<td>6/7</td>
<td>1/7</td>
<td>0/7</td>
<td>0/7</td>
</tr>
<tr>
<td>Small intestine</td>
<td>0/13</td>
<td>4/6</td>
<td>0/6</td>
<td>0/6</td>
<td>2/6</td>
</tr>
<tr>
<td>Cecum tonsil</td>
<td>2/13</td>
<td>4/6</td>
<td>0/6</td>
<td>0/6</td>
<td>2/6</td>
</tr>
<tr>
<td>Gonad</td>
<td>6/13</td>
<td>4/4</td>
<td>0/4</td>
<td>0/4</td>
<td>0/4</td>
</tr>
<tr>
<td>Peripheral nerve</td>
<td>0/13</td>
<td>0/5</td>
<td>0/3</td>
<td>0/3</td>
<td>3/3</td>
</tr>
<tr>
<td>Central nervous system</td>
<td>0/4</td>
<td>0/4</td>
<td>1/4</td>
<td>0/4</td>
<td>3/4</td>
</tr>
</tbody>
</table>

a) Incidence of tumor-like lesions of 13 chicks diagnosed as having LL. b) Number of chicks with lesions/number of chicks affected. c) Fibroma. d) Edema. e) Not examined. f) Perivascular cuffing.

related to ascites described below were recognized in the liver, heart, and lungs of chicks of this group. Right-side cardiac enlargement, and variable liver changes were macroscopically observed. A large amount of ascitic fluid with or without fibrin clots accumulated in the hepatic peritoneal cavity, and excess gelatinous fluid in the pericardial sac was present. Some chicks with ascites had small white spot lesions in the heart, and the apex of the heart was blunt with dimples. Their heart walls were thin and inelastic. The lungs were often congested and edematous, and the liver, kidneys, and spleen were congested. The livers in affected chicks were sometimes shrunken with a grayish capsule. The capsule of the liver was greatly thickened (Fig. 3B). Except the bursa, tumor-like lesions were macroscopically recognized in the liver, spleen, kidneys, heart, thymuses and bone marrow at a low frequency. Atrophy of the thymuses was macroscopically observed in two chicks. Histopathologic changes of LL
Fig. 2. Microscopic lesions of an LL-chicken (Chicken No. 805). A: Proliferation of tumor cells in the follicle of bursa. HE, × 80. B: Focal proliferative lesions with clear boundaries to normal tissue of kidney. HE, × 80. C: Monomorphic large lymphoid cells in tumorous tissue of liver. HE, × 750. D: Monomorphic large lymphoid cells in tumorous tissue of bursa. In nucleus, a chromatin network and a nucleolus are evident. HE, × 750.

were almost consistent with those of the macroscopic observation. However, lymphocytic infiltration was frequently observed in the heart (Fig. 4) and lungs, and in the liver, bone marrow, proventriculus and peripheral nerves with low frequency. Lymphocytic infiltration in the CNS (perivascular cuffing) was frequently observed. Atrophy of the thymuses was microscopically observed in two chicks.

Twenty-one chicks (35.6%) were macroscopically diagnosed as having ascites. In addition to ascitic lesions, atrophy of the bursa and thymuses was macroscopically observed in 13 and 12 of the 21 chicks, respectively. Two of the 21 chicks were examined for incidence of histopathologic changes in various organs. Lymphocytic infiltration was observed in the liver, heart, lungs and proventriculus. Microscopic changes of serous exudate were observed in the liver, heart and lungs of the two chicks examined. In addition to ascitic lesions, one chick had LL lesions in the bursa and the other had only ascitic lesions.

Two cases were diagnosed as erythroblastosis. One of them had microscopic LL lesions in the bursa, heart and kidneys, lesions of myelocytosis in the kidneys and bone marrow, perivascular cuffing of CNS and lymphocytic infiltration in the heart and peripheral nerves.

Of the 12 chicks classified as others, 8 showed similar macroscopic changes such as mild swelling of the liver. White spot lesions in the heart were observed in 2 chicks, atrophy of the bursa in 2 chicks, and atrophy of thymus in one chick out of the 8 chicks. White spot lesions in the bone marrow of 2 chicks out of these 7 chicks were also observed. LL lesions were observed in the heart, bursa, bone marrow and proventriculus of one chick. Lymphocytic infiltration was observed in all of the liver and
proventriculus, as well as in the heart, bone marrow and CNS of one chick. Of the remaining 4 chicks, two survived without any clinical signs until the end of the observation period of 102 days in Exp. 1 and they had no gross lesions in the visceral organs. The remaining two chicks only showed weakness, and atrophy of the bursa and white spot lesions in the heart were observed in these chicks. One of them had microscopic LL lesions in the bursa and lymphocytic infiltration in the heart and proventriculus.

Detection of MDV or REV: Serotype 2 MDV was detected in the concentrated viral inoculum. The virus titer in the inoculum was 10 PFU per ml. Serotype 2 MDV was detected in 5 out of 8 chicks in Exp. 4. REV was detected neither in the concentrated viral inoculum nor in the 8 samples collected at sacrifice in Exp. 4.

Examination of antibodies against MDV and REV: Thirty-six sera, seven from Exp. 1, eight from Exp. 2, 13 from Exp. 3 and 8 from Exp. 4, collected from chicks at sacrifice were examined for antibodies against MDV or REV by AGP test or ELISA. Neither MDV antibody nor REV antibody was detected in any of the sera examined.

DISCUSSION

Inoculation of a viral material (culture fluid) from an LL-cell line mainly induced ascites, but LL was not induced in any chicks inoculated. On the other hand, the chicks inoculated with the concentrated viral material developed LL, complication of ascites with LL, ascites, erythroblastosis, and lymphocytic foci possibly related to serotype 2 MDV infection, during the short incubation period. Here, we discuss the pathogenesis of LL, ascites, and lymphocytic foci possibly related to serotype 2 MDV infection.

LL: In general, LL appears between the 14th and 30th weeks of age, and incidence is usually highest at about sexual maturity [18, 19, 27]. In our present experiments, however, the incubation period of LL to onset was remarkably shortened in chicks inoculated with the concentrated viral material, and ranged from 27 to 74 dpi (GM: 48.4 days). Why was LL induced rapidly in chicks inoculated with the concentrated viral material? One possibility is that the virus inoculum contains a low amount of LL-specific virus which has viral oncogene(s). However, this possibility is unlikely, because such a virus has not been isolated from rapidly-induced LL tumors until now. Another possibility is that the effectiveness of insertion of...
long terminal repeats (LTR) of ALV near the c-myc gene of B cells [10, 13] is raised by inoculation with a large amount of ALV. Even though it is possible, however, it would not be enough to induce LL rapidly, although it would be enough to induce follicular hyperplasia [1, 11, 26]. In the present experiments, atrophy of bursa, except LL cases, was frequently observed in chicks inoculated with the concentrated viral material, atrophy of thymuses was sometimes recognized in them, and no antibody against serotype 2 MDV was detected in any sera of chicks inoculated with the concentrated viral material. These results indicate the possibility that the chicks inoculated with a large amount of ALV were in an immunosuppressed condition. Therefore, it may be suggested that not only inoculation with a large amount of ALV, which induces follicular hyperplasia and formation of the bursal tumors, but also immunosuppression induced by ALV, probably necessary for tumor progression, are essential for the rapid induction of LL.

Burne stri et al. [6] reported that high doses of RPL-12 ALV mainly induced erythroblastosis, whereas doses close to the endpoint predominantly induced LL. However, in our present experiments low doses of ALV mainly induced ascites and high doses of ALV from the LL-cell line mainly induced LL and ascites. The incidence of erythroblastosis was very low (3.4%), and in our previous data [15], the virus released from an established cell line induced some kinds of tumors in chicks after a long incubation period, but never achieved such rapid LL and as reported in present experiments. Therefore, it seems very important to investigate whether the rapid induction of LL is a specific attribute of the virus for the specific cell line or not.

Studies to characterize further the ALV isolated from the LL-cell line, in vivo and in vitro are in progress.

The incidence and distribution of macroscopic and microscopic lesions of LL in the organs in the present experiments were almost the same as those observed previously [18, 19, 27], except for lymphocytic infiltration in CNS. Lymphocytic infiltration in CNS may be caused by synergistic effects of ALV and serotype 2 MDV released from the LL-cell line and included in the inoculum [14], since such lesions were not induced in chicks inoculated with ALV alone or serotype 2 MDV alone, both of which had been isolated from the inoculum in the present experiments.

Bacon et al. [2] reported that the serotype 2 MDV infection augments the incidence of LL in LL susceptible chicken lines. However, it is not clear if such augmentation is involved in rapid induction of LL or in the incidence of LL in the present studies. More work is needed before either of these possibilities can be confirmed.

Ascites: Ascites secondary to right ventricular failure (ARVF) occurs worldwide in young broiler chickens and is a significant cause of mortality in many flocks [28]. Maxwell et al. [25] reported that avian tumor virus like particles were seen between myocardial fibers and in other visceral organs in an ultrastructural study of chickens affected with ARVF but not in the tissues of unaffected control chicks. However, a relationship between the virus and ARVF was not established. Gilka and Spencer [12] reported that most of SPF chickens inoculated with RAV-1 had focal chronic lymphocytic or lymphoplasmacytic myocarditis, and they suggested that the myocardial lesions observed might be responsible for chronic circulatory syndrome (CCS) characterized by right-sided heart failure. These reports suggest a possibility that ALV is a causal agent of ARVF.

The clinical signs and autopsy findings of chicks diagnosed as having ascites in the present studies closely resembled those reported earlier [28], except for the white spot lesions in the heart. In our previous experiments [18, 19], ascites was also induced in chicks of both line 151 and line BK when inoculated with ALV subgroup A, although the incidence of the ascites was not so high (4–22%) and the incubation period was not so short (64–149 dpi).

In the present experiments, ascites was observed in 9 out of 15 chicks (60.0%) 59–119 dpi inoculated with the culture fluid, and in 33 out of 59 chicks (54.2%) 32–83 dpi inoculated with the concentrated ALV material. Eleven of them developed complications with LL. Some of those chicks had white spot lesions in the heart, and lymphocytic infiltration was microscopically observed in their hearts. Most of the heart walls of these chicks were thin and inelastic. So, it is considered that in the chicks with ascites, heart dysfunction occurred first leading to the dysfunction of circulation of the blood, and finally to the accumulation of ascitic fluid in the hepatic peritoneal cavities and in the pericardial sac. Our recent studies suggest that the high incidence of ascites in the present studies is not due to serotype 2 MDV included in the viral inocula but due to the properties of the inoculated ALV.

Others: Of the 8 chicks with similar macroscopic lesions, 3 were histopathologically examined. Lesions like those observed in classical MD [8, 35], such as perivascular cuffing in CNS were recognized in one chick. Schat and Calnek [31] reported that serotype 2 MDV did not induce MD tumor lesions even in immunosuppressed chickens, and that SB-1 strain (serotype 2 MDV) can induce a lytic infection resulting in cell death, and therefore, degenerative lesions, but it reaches pathogenic levels only when the host is immunologically incompetent. So, these results suggest that the mild lymphocytic foci in peripheral nerves and perivascular cuffing in CNS observed may have been induced by serotype 2 MDV infection in chicks in immunosuppressive conditions caused by the ALV infection.

Our present findings suggest that the rapid induction of LL and ascites provides a useful system not only for analyzing LL-lymphomagenesis, especially for oncogene(s) involved in LL-lymphomagenesis, but also for studying the pathogenesis of ascites, and the interaction between retrovirus and herpesvirus.

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RAPID INDUCTION OF LL AND ASCITES

