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CHARACTERIZATION OF TRANSPLANTABLE SUBLINE MDCC-MSB1-CLO. 18 DERIVED FROM MDCC-MSB1

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MDCC-MSB1-Clo. 18 subline, derived from a Marek's disease (MD) lymphoblastoid cell line MDCC-MSB1, was highly transplantable and lethal for chickens. A dose-response relationship was observed between the number of tumor cells inoculated and the development of tumors in the recipient chickens.

In addition to lymphoma formation at the site of transplantation, the recipients developed metastatic lesions in various visceral organs. Age resistance of the chickens to the tumor development was also observed. No tumor development was observed in nude mice inoculated with MDCC-MSB1-Clo. 18 cells. MD virus was isolated from duck embryo fibroblasts inoculated with MDCC-MSB1-Clo. 18 cells and also from kidney cell cultures of MDCC-MSB1-Clo. 18-inoculated chickens. MDCC-MSB1-Clo. 18 cells reacted with anti-B_E and B_K sera in the membrane immunofluorescence test. The tumor cells collected from the chickens also reacted with anti-B_E and B_K sera, while the erythrocytes from these chickens did not react with these antisera. This indicated that the tumors were originated from the inoculated cells.

Key words: MDCC-MSB1, MDCC-MSB1-Clo. 18, lymphoblastoid cell line, Marek's disease, transplantable cell line

INTRODUCTION

Marek's disease (MD), a contagious lymphoproliferative disease of chickens caused by a herpesvirus, MD virus (MDV),⁴⁾ is characterized by lymphoma formation in many visceral organs and infiltration of lymphocytes in the peripheral nerves.²⁾ Many cell lines have been established from primary virus-induced tumors^{1,15,20)} or from tumor transplants originally established by inoculation of the chicken with MDV.^{3,8,13)}

All cell lines cause tumor formation in susceptible chickens; some cause a primary

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virus-induced tumor while others produce transplantable tumors. The cell lines derived from primary tumors are usually not transplantable, but most of those derived from tumor transplants are transplantable to chickens.^{6,8,13,20)}

MDCC-MSB1 (MSB1-O) cell line is derived from a splenic lymphoma of chicken inoculated with BC-1 strain of MDV.¹⁾ The cell line produced MDV and had oncogenicity in chickens but was not originally transplantable.⁶⁾ Recently, Matsuda et al. described the establishment of a transplantable subline, MDCC-MSB1-41C (MSB1-41C) cell line, from the original MSB1-O cell line, and the subline was highly transplantable and lethal for chickens.^{10,11)} The MSB1-41C is the first transplantable cell line derived from the original non-transplantable cell line. In the present report, we described another transplantable subline, MDCC-MSB1-Clo. 18 (MSB1-Clo. 18), derived from the MSB1-O cell line, and discussed about the differences between the characteristics of MSB1-41C and MSB1-Clo. 18.

MATERIALS AND METHODS

Cell lines

MSB1-Clo. 18 cell line was derived from MSB1-O by cloning three times in soft agar culture as described by Akiyama and Kato.¹⁾ MSB1-Clo. 2, Clo. 15 and Clo. 16 sublines were also derived from MSB1-O cell line. These cells were cultured in the same manner as described previously.⁹⁾

Intraperitoneal inoculation of the cells to chickens

MSB1-Clo. 2, Clo. 15, Clo. 16 or Clo. 18 (5×10^7 viable cells/bird) and MSB1-O (3×10^7 viable cells/bird) were inoculated into each of two 6-day-old chickens. MSB1-Clo. 18 was also inoculated into 5 one-day-old chicks at a dose of 3×10^7 viable cells/bird.

All surviving chickens were killed on 22 days post inoculation (PI).

Subcutaneous inoculation of the MSB1-Clo. 18 cells to chickens

MSB1-Clo. 18 cells (1×10^7 viable cells/bird) were subcutaneously inoculated into the wingwebs of 6 one-day-old chicks. The length (l), width (w) and height (h) of tumors at the inoculation sites were measured and the volume (V) of the tumors was calculated by means of the formula for the volume of a hemiellipsoid, the form most nearly approximating the shape of the tumors: $V = 0.5236lwh$.⁵⁾

Relation between the number of inoculated cells and the growth of tumor

MSB1-Clo. 18 cells at a dose of 1×10^5 , 5×10^5 , 1×10^6 , 5×10^6 or 1×10^7 cells per chick were subcutaneously inoculated into the wingwebs of each of 5 one-day-old chicks. The tumor growth in the chickens were observed for 35 days. All surviving chickens were killed on 35 days PI. The 50% lethal dose (LD_{50}) for the MSB1-Clo. 18 cell line was calculated according to the method of Reed and Muench.¹⁶⁾

Relation between the age of chickens and the growth of MSB1-Clo. 18 cells in chickens

Each of five chickens from four different age groups was inoculated subcutaneously

with MSB1-Clo. 18 cells (1×10^7 viable cells/bird) into the wingwebs. The four groups consisted of 1-, 7-, 14- or 21-day-old chickens. Three weeks after inoculation, the chickens were examined for the tumor growth.

Virus isolation

Duck embryo fibroblast (DEF) cultures used for virus isolation from MSB1-Clo. 18 cells were grown in Eagle's minimum essential medium supplemented with 10% calf serum at 37°C in an atmosphere containing 5% CO₂. MSB1-Clo. 18 cells (4×10^6 cells/35 mm plate) were inoculated onto the DEF monolayers.

The appearance of plaques caused by MDV was considered as an indicator of virus isolation.

Detection of anti-MDV antibodies

The sera collected from 60 chickens inoculated with MSB1-Clo. 18 cells and 5 contact-infected chickens were examined for the presence of anti-MDV antibodies by agar-gel-precipitation (AGP) test¹⁴⁾ and enzyme-linked immunosorbent assay (ELISA).²¹⁾

Inoculation of MSB1-Clo. 18 cells or transplantable tumor into athymic nude mice

BALB/c-nu/nu mice 4 to 8 weeks of age were used in this experiment. Two mice were subcutaneously and two were intraperitoneally inoculated with 3×10^7 viable MSB1-Clo.18 cells. Four cyclophosphamide (Cy)-treated mice were also inoculated with the same number of the cells as above. Cy-treatment was done twice by intraperitoneal injection of Endoxan (Shionogi & Co. Ltd., Osaka, Japan) per mouse on 2 and 3 days prior to the inoculation of MSB1-Clo. 18 cells.

Transplantable tumor cells from chickens inoculated with MSB1-Clo. 18 cells (0.1 ml of the cells packed at $600 \times g$ for 5 minutes) were inoculated into 8 nude mice in the same manner as described above.

The mice were killed on 21 days PI and the tumor development was examined.

The reactivity of MSB1-Clo. 18 cells with antisera specific to B complex antigens

The reactivity of MSB1-Clo. 18 cells with alloantisera against chicken major histocompatibility antigens (MHA) (B complex antigens) was examined by membrane immunofluorescence (MIF) test by using 13 antisera to MHA at the serum dilution equal to the agglutination titer as described previously.⁹⁾

RESULTS AND DISCUSSION

The cells from MSB1-O cell line and four MSB1 sublines (MSB1-Clo. 2, Clo. 15, Clo. 16 and Clo. 18) were inoculated intraperitoneally into 6-day-old chickens at the dose of 3×10^7 or 5×10^7 cells per bird. Two chickens inoculated with MSB1-Clo. 18 cells died on 13 and 14 days PI with tumor development in the peritoneal cavity. However, none of other chickens died although tumor developed during the experimental period of 22 days. In another experiment, all of the five chickens intraperitoneally inoculated with 3×10^7 of MSB1-Clo. 18 cells died between 15 and 18

days PI. At the necropsy, tumor mass was observed in the peritoneal cavity of these birds. By subcutaneous inoculation of MSB1-Clo. 18 cells into the wingwebs of one-day-old chicks, tumor development was observed in all birds. The growth curves of the tumors are shown in fig. 1. The tumors grew rapidly after 10 days PI, and the birds began to die on 19 days PI. All chickens died within 24 days PI. Tumor developments were reported in 40% of the chickens inoculated with MSB1-41C via the wingweb and tumor-carrying birds were still alive on 40 days PI.¹¹⁾ The results obtained in the present experiment suggest the higher transplantability and lethality of the MSB1-Clo. 18 cells than those of MSB1-41C cells. The *in vivo* characteristics of MSB1-Clo. 18 cells were further investigated by the subcutaneous inoculation of the cells into wingwebs of chickens.

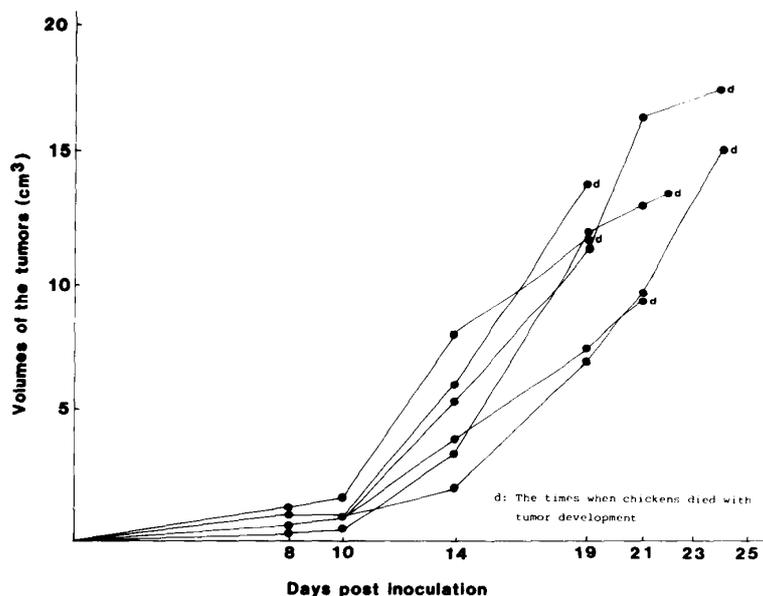


FIGURE 1 Growth curves of the tumors after subcutaneous inoculation with MSB1-Clo. 18 cells (1×10^7 cells/bird) into wingwebs of one-day-old chicks

To examine the dose effect of MSB1-Clo. 18 cells on the tumor growth, various numbers of cells were inoculated into chickens. The results are shown in table 1. All chickens inoculated subcutaneously with more than 5×10^6 cells developed tumor while none of the chickens inoculated with 1×10^5 cells developed tumor. The LD_{50} of MSB1-Clo. 18 in this experiment was 1.3×10^6 cells per bird. In many cases, the dead chickens had gross metastatic lesions in various organs (table 2). In contrast, no metastatic lesions were observed when the cells were intraperitoneally inoculated into the chickens as described above. The occurrence of metastasis was reported in the chickens inoculated intraperitoneally with MSB1-41C cells.¹⁰⁾ This discrepancy

TABLE 1 *Effect of the number of inoculated cells on the growth of MSB1-Clo. 18 cells in vivo*¹⁾

NUMBER OF INOCULATED CELLS	TUMOR-POSITIVE CHICKENS	INOCULATED CHICKENS	%
1×10^7	5/5		100
5×10^6	5/5		100
1×10^6	1/5		20
5×10^5	1/5		20
1×10^5	0/5		0

- 1) The cells were inoculated subcutaneously into the wingwebs of one-day-old chicks. They were observed for 35 days after inoculation.

TABLE 2 *Metastasis in chickens inoculated with MSB1-Clo. 18 cells*¹⁾

TISSUES OR ORGANS WITH METASTATIC LESIONS	TUMOR-POSITIVE CHICKENS	%
Kidney	18	90
Lung	12	60
Liver	12	60
Stomach	4	20
Heart	3	15
Spleen	1	5
Ovary	1	5
No metastasis	1	5

- 1) Twenty chickens were inoculated subcutaneously with MSB1-Clo. 18 cells (1×10^7 cells/bird) into the wingwebs at one day of age. They were observed for 21–35 days. Necropsy was carried out at the time of death to examine for metastasis.

might be caused by the difference of the dosage of inoculated cells. The dosage used in the present experiments was 3×10^7 cells/bird which was three times higher than that of MSB1-41C cells used in the previous report.^{10,11)} It seems that the bird which received the higher dose of cells died before they showed metastatic lesions. Ninety percent of the chickens subcutaneously inoculated with MSB1-Clo. 18 cells had metastasis in the kidneys. The normal kidney cells of these chickens were often replaced by tumor cells. In contrast, only 29% of the chickens inoculated intraperitoneally with

MSB1-41C cells had metastatic lesions in the kidneys.¹⁰⁾ Previously, the selection of a variant which showed specific ability to metastasize to liver from MDCC-RP1 cell line had been reported.¹⁸⁾ Furthermore, a cell surface antigen which was correlated with specific liver metastasis was detected on this variant.¹⁷⁾ The difference of the tendency on metastatic lesion between MSB1-41C and MSB1-Clo. 18 might be caused by existence of such cell surface antigen(s). Further study is necessary to clarify on this point.

To examine the relation between transplantability of MSB1-Clo. 18 cells and the age of the recipient chickens, chickens of various ages were inoculated with MSB1-Clo. 18 cells. The results are shown in table 3. All chickens inoculated with the cells at one day of age had tumors. The number of tumor-positive chickens decreased as the age of the recipient chickens increased. These results are essentially similar to those reported¹⁰⁾ and suggest that the tumor development is inhibited by host immune mechanism developing with age of chickens.

TABLE 3 *Relation between the age of chickens and the growth of MSB1-Clo. 18 cells in vivo*¹⁾

AGE OF THE CHICKENS AT THE TIME OF INOCULATION	TUMOR-POSITIVE BIRD / INOCULATED BIRD	%
One day	5/5	100
One week	4/5	80
Two weeks	1/5	20
Three weeks	0/5	0

- 1) MSB1-Clo. 18 cells (1×10^7 viable cells/bird) were inoculated subcutaneously into the wingwebs of the chickens at each age. The inoculated chickens were observed for 21 days after inoculation.

MDV was isolated by the direct kidney culture prepared from kidneys of chickens inoculated with MSB1-Clo. 18 cells or by inoculation of MSB1-Clo. 18 cells in the DEF cultures. Antibodies against MDV were detected by ELISA in all sera tested but not by AGP test. These results suggest that MDV could be rescuable from MSB1-Clo. 18 cells.

No tumor development was observed in the nude mice under any condition examined in the present experiment.

The results of the reactivity of MSB1-Clo. 18 cells with 13 specific antisera against MHA of chicken B system are shown in table 4. The percentage of MIF-

TABLE 4 Reactivities of 13 specific antisera to histocompatibility antigens of chicken B system with MSB1-Clo. 18 cells¹⁾

ANTISERUM SPECIFIC TO B COMPLEX ALLOANTIGENS	AGGLUTINATION TITER (2 UNITS) TO HOMOLOGOUS ERYTHROCYTES	MIF-POSITIVE PERCENT
A	2	4.5
B	4	0.9
C	2	8.7
D	2	5.6
E	2	48.4
G	2	0.8
I	2	0
K	2	95.2
L	2	0.5
M	2	8.9
N	3	0.9
O	2	1.7
T	2	0

- 1) The reactivities were examined by membrane immunofluorescence (MIF) test using 13 antisera at the serum dilution equal to the agglutination titer.

positive MSB1-Clo. 18 cells with anti-B_E and B_K sera were 48.4 and 95.2, respectively but were below 10 with the remaining eleven antisera. The reactivity of MSB1-Clo. 18 cells with anti-B_E and B_K sera was similar to that of MSB1-O, Clo. 2, Clo. 15 or Clo. 16 cells, but different from that of MSB1-41C cells.⁹⁾ MSB1-41C cells reacted with anti-B_D, B_M or B_E sera but the percentage of positive cells was less than 20. They did not react with anti-B_K serum⁹⁾. We speculated previously that transplantability was related to the degree of MHA expression of the donor cells.⁹⁾ The present results, however, indicated that the possibility is rather low. Theis et al.¹⁹⁾ reported that transplantable MD lymphoma from B¹/B¹ chickens (MDCT-NYM1) grew progressively in all injected syngeneic and hybrid B¹/B² chickens, although it was not transplantable to allogeneic B²/B² chickens. On the other hand, Fabricant et al.⁷⁾ reported that transplantable MDCT-CU7 cells developed progressive tumors in the S-strain chickens which did not share MHAs with the inoculum cells but were genetically susceptible to MD. In the present experiment, MSB1-Clo. 18 developed tumors in allogeneic chickens. The tumor cells collected from the chickens also reacted with anti-B_E and B_K sera, while the erythrocytes from these chickens did not

react with these antisera. Furthermore, MSB1-Clo. 18 developed tumors in commercially-reared one-day-old chickens (data are not shown) as well as in specific-pathogen-free chickens, although both lines of chickens were not so susceptible to MD as S-line chickens. These results suggest that the transplantability of MSB1-Clo. 18 was not related to the MHA nor genetical resistance of the chickens. The exact mechanism of transplantability of MSB1-Clo. 18 and MSB1-41C should be examined more minutely.

It is interesting to note that transplantable variants such as MSB1-41C and MSB1-Clo. 18 originated from the non-transplantable MSB1-O cell line. The mechanism involved in the selection of the variants is not known. To investigate and compare the characteristics of such transplantable variants may be important to analyze the mechanism of the transplantability of MD tumor cells.

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