Suppression of IgM Production and its Mechanism in Chicks Transplanted with Marek's Disease Lymphoma Cell Lines

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The visceral type of Marek's disease (MD) in chicken is one of lympho proliferative diseases of T cell origin caused by MD virus. It has been known that this disease is accompanied with various phenomena of acquired immunodeficiency [1, 5, 10, 11].

Suppression of IgM production is especially remarkable among these phenomena [6, 7, 8]. To clarify the mechanism of reduced IgM production in the case of MD, it is necessary to elucidate the functional relationship between T and B lymphocytes of chickens.

In the present experiment, chicks were transplanted with MD lymphoma cells was examined for the mechanism of reduced IgM production.

Three transplantable T lymphoma cell lines derived from lymphoma induced by MD virus in Black Minorca strain of chickens (BM-C strain with major histocompatibility antigen, genotype B^A/B^A) [9] were used. As summarized in Table 1, both the BMC-TL1 cell line (a cloning line) and BMC-TL2 cell line [3, 9] were negative for MD associated tumor surface antigen (MATSA) by immunofluorescence. These two cell lines were settled alive in a region under the wing after transplantation. Glossly, the BMC-TL1 cell line metastasized mainly to the liver, whereas the BMC-TL2 cell line did mainly to the spleen. In contrast, the BMC-L2-1 line [2, 9], derived from cloned MATSA-positive cell line cultured by *in*

vitro passage, continued to grow in a region under the wing after transplantation, but did not metastasize to any other organ.

Each of these MD lymphoma cell lines ($0.5-1.0 \times 10^7$ cells) was transplanted in to the wings of three-days-old chicks of Black Minorca and examined for serum IgM levels by immunoelectrophoresis and immunodiffusion test with anti-chicken whole serum and anti-chicken μ chain at two weeks after transplantation.

In immunoelectrophoresis, no precipitation line of IgM was detected in serum of chicks transplanted with the BMC-TL1 or BMC-TL2 cell line (Fig. 1).

The results of estimation of serum IgM in the chicks transplanted with MD lymphoma cell lines is shown in Table 2 and Fig. 2. In immunodiffusion analysis, a precipitation line of IgM was not detected or slightly detected in the serum of chicks transplanted with the BMC-TL1 or BMC-TL2 cell line (Fig. 2). All the chicks with metastasis of lymphoma cells of the BMC-TL1 and BMC-TL2 cell lines to the visceral organ, were found to have distinctly reduced IgM in serum. In contrast, in the chicks transplanted with BMC-L2-1 line, lymphoma cells proliferated at the site of transplantation, but did not metastasize to any other visceral organ, and serum IgM was not reduced (Table 2).

To further confirm the suppression of IgM production in the chicks transplanted with the BMC-TL2 cell lines, their splenic cells were examined for ability to produce specific anti-

Table 1. Characteristics of transplantable MD lymphoma cell lines

Cell line	MATSA	Transplantable ratio (wing)	Metastasized organs	Ratio of metastasis
MDTC-BMC-TL1 ^{a)}	negative	100.0%	liver	80.0%
MDTC-BMC-TL2 ^{a)}	negative	95.3%	spleen	100.0%
MDCC-BMC-L2-1 ^{b)}	positive	90.4%	negative	0.0%

a) Transplanted tumor cell line derived from MD lymphoma [12].

b) Cultured tumor cell line derived from MD lymphoma [12].

Fig. 1. Immunoelectrophoretic analysis of chick sera. A: Anti-chicken serum, B: anti-chicken μ chain, 1: Serum of normal chick, 2: Serum of chick transplanted with MD lymphoma cells (The precipitation line of IgM was not detected.)

Table 2. Serum IgM in chicks transplanted with MD lymphoma cell lines

Transplanted cell lines	No. of chicks	Degrees of serum IgM ^{a)}			
		++	+	+	-
Normal control	10	10	0	0	0
BMC-TL1	10	0	0	3	7
BMC-TL2	10	0	0	1	9
BMC-L2-1	10	8	2	0	0

a) Graded into four degrees on the basis of the strength of precipitation lines exhibited by the immunodiffusion analysis (++: normal standard, +: slightly reduced, -: remarkably reduced, -: negative).

sheep red blood cell IgM plaque forming cells (anti-SRBC IgM PFC). This was estimated by the method of Hirota *et al* [4] at five days after the administration of 20% SRBC (1.0 ml). The ability to produce the anti-SRBC IgM PFC was suppressed so distinctly that it was not detectable (10 cases). In contrast, the values of IgM PFC of normal control chicks were $558 \pm 167/10^6$ splenic cells (10 cases).

From the results, there was relationship among the phenomena (metastasis, IgM reduction and presence of MATSA) regarding MD tumor. It was also assumed that the suppressions of the IgM production and the specific antibody to SRBC might have been induced by the suppression of the differentiation and maturation of IgM producing B lymphocytes in chicks transplanted

Fig. 2. Immunodiffusion analysis of chick sera. X: Anti-chicken μ chain, a: Serum of normal chick (Degree of IgM: ++), b: Serum of chick transplanted with MD lymphoma cells (Degree of IgM: +), c: Serum of chick transplanted with MD lymphoma cells (Degree of IgM: -).

Table 3. Serum IgM in chicks adoptively transferred with splenic lymphocytes of chicks transplanted with BMC-TL1 cells

Group	No. of chicks	Degrees of serum IgM ^{a)}			
		++	+	+	-
Control	10	10	0	0	0
Transplanted	10	0	0	3	7

a) See Table 2.

with the MD lymphoma cell lines.

An experiment was carried out to elucidate the role of splenic lymphocytes in the mechanism of reduced IgM production in chicks transplanted with the MD lymphoma cell lines. The splenic lymphocytes were collected from chicks transplanted with the BMC-TL1 cell line at two weeks after the transplantation. These cells were non-lymphomatous microscopically, and composed mostly of T cells by the fluorescent antibody test. They were administrated intraperitoneally to one-day-old chicks of the same breed ($1-2 \times 10^7$ cells). Serum IgM was estimated by the immunodiffusion analysis in these chicks 10 days after the transplantation. As shown in Table 3, the serum IgM was suppressed remarkably in all the cases. In the normal control chicks of the same age administered with normal splenic lymphocytes, however the serum IgM was not reduced in all cases.

These results indicated that immunosuppres-

sive lymphocytes were predominant in the spleen of chicks transplanted with the metastatic MD lymphoma cell line, and that those lymphocytes might play an important role in the mechanism of reduced IgM production in the chicks affected with the MD lymphomatosis.

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要 約

マレック病腫瘍細胞株移植ひなにおける IgM 産生抑制とその機構（短報）：小長谷勝利・大木与志雄（日本獣医畜産大学生理化学教室）——マレック病（MD）関連腫瘍表面抗原（MATSA）陰性、転移性の MD 腫瘍細胞株を移植したひなにおいては、血清 IgM の減少および羊赤血球に対する特異的 IgM PFC 産性能の抑制が認められた。また、MD 腫瘍細胞株移植ひなの脾臓細胞を移植したひなにおいても同様な免疫抑制が認められた。これらの結果から、MD 腫瘍細胞株移植ひなにおいては、免疫抑制性リンパ球が優位になっていると考えられた。