Expression of Major Histocompatibility Antigens on Marek's Disease Lymphoma-derived Cell Lines

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Expression of major histocompatibility antigens (MHAs) on six cell lines derived from Marek's disease (MD) lymphomas was investigated by the membrane immunofluorescence test. The results revealed varying expression of MHAs on the cell lines tested (MDCC-JP1, JP2, HP1, HP2, RP1 and MSB1). Antigenic diversity of MHAs was observed even among four sublines and 18 clones derived from the MSB1 cell line, indicating that this cell line consists of a mixture of cells expressing different MHAs. Furthermore, after more than six months' cultivation, a change and/or a reduction of the reactivity with antisera specific for antigens of the B complex were observed in some of these MD cell lines. These results suggested the possibility of changes in MHA expression on MD lymphoma line cells during long-term cultivation *in vitro*.—*Key words:* Lymphoma cell line, Major histocompatibility antigen, Marek's disease.

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Since the cell membrane is viewed as a primary site of cell-cell interaction, characterization of membrane changes resulting from malignant transformation may give an insight into the mechanism of the cell-mediated immunological defense against tumor cells. In human and murine systems, the effects of malignant transformation on cell surface structure coded by genes in the major histocompatibility complex (MHC) have been investigated. It is shown that virus-transformed cells may carry HLA or H2 antigens not demonstrated on normal cells [4, 28]. It is also shown that these cells may lack the major histocompatibility antigen (MHA) expressed on normal cell surfaces [15, 31]. Furthermore, several investigators have reported the presence of additional HLA determinants on human lymphoid cells after short and long-term culture [12, 20].

The chicken MHC (B complex) remains to-date the best analyzed MHC outside mam-

mals. The relation between the chicken MHC and virus infections has been investigated [3, 17], since genetically determined resistance to infection is of economic importance in the poultry industry. Marek's disease (MD), a contagious lymphoproliferative disease caused by a herpesvirus [9, 24], is one of the most important diseases of the chicken. Several cell lines have been established from MD lymphomas [1, 7, 25, 29, 35]. The cells of these cell lines express MD tumor-associated surface antigens (MATSAs) [25, 29, 33]. MHAs have also been demonstrated on these cells [6, 23, 35]. Evidence for the independence of MATSA and MHA on MD line cells has been presented [10, 23]. However, comparisons between the MHAs on several MD cell lines and the possibility of its changes in expression during *in vitro* cultivation have not yet been investigated.

In the present communication, we report the expression of MHA on the cells of several

MD lymphoma cell lines and its change during long-term culture.

MATERIALS AND METHODS

Cell lines and cell cultures: Six MD lymphoma cell lines and four sublines derived from one of the cell lines were used for the present study. The nomenclature used for the cell lines is that proposed by Witter *et al.* [34]. MDCC-HP1 and MDCC-HP2 were derived from ovarian lymphomas of Rhode Island Red chickens [29]. MDCC-RP1 was derived from the JMV transplant [25] and MDCC-JP1 and MDCC-JP2 were derived from ovarian lymphomas of inbred chickens [35]. MDCC-MSB1 was derived from a splenic lymphoma [1]. Four sublines and 18 clones derived from MSB1 were used. The four sublines were the original MSB1 cultured for less than one year (MSB1-0), MSB1 cultured for more than three years in our laboratory (MSB1-E), transplantable subline MSB1-41C [21] and MSB1-33C cultured at 33°C [2]. Eighteen clones were derived from MSB1-0 by cloning in soft agar in the same manner as described previously [1]. All MD cell lines and MSB1 sublines or clones used in the present studies reacted with monospecific antisera to MATSA (unpublished data).

These cells were cultured in RPMI 1640 supplemented with 10% fetal calf serum, 200 units/ml of penicillin G potassium, 200 µg/ml of streptomycin sulfate and 50 units/ml of amphotericin B. Except for MSB1-33C, the cells were cultured at 41°C in an atmosphere containing 5% CO₂.

Antisera: Antisera specific to B complex antigens were prepared by alloimmunization as described previously [22]. After extensive absorption of antisera (diluted to 1:5) with packed chicken erythrocytes for monospecificity, specificity of the antisera was examined by hemagglutination and confirmed by the lack of cross-reactivity to heterologous erythrocytes. The absorbed antisera are presumably directed against class I MHC molecules

and are specific for a particular allele of the strains of chickens maintained in the Animal Breeding Laboratory of Hiroshima University. Agglutination titers to homologous erythrocytes described in tables are expressed as dilutions (2 units) of antisera usually used for hemagglutination. Therefore, the estimated titers of these antisera to homologous erythrocytes can be obtained by multiplication by 10. The details of the methods have been described previously [22].

The nomenclature of the B alleles used in the present paper was given by Matsumoto and Okada [22]. The international comparison tests for blood grouping of chickens, including B system, were carried out by using antisera prepared by Okada (Japan) and McDermid (England) [26]. The blood group antigens of B_A (Okada's classification) and B₁₁ (McDermid's classification); and B_G and B₉ are comparative. Some similarities existed between the antigens of B_{BM} and B₂; B_C and B₆; B_E and B₁₄; B_G and B₇, B₃₈; B_K and B₁₃; B_L and B₁, B₁₄; B_M and B₁₂, B₁₉; and B_{MO} and B₁, B₂, B₈. The comparison test between the nomenclature of B system classified by Okada and that classified by Briles has not yet been made in detail. But in the preliminary experiments, some similarities were observed between B_N (Okada's classification) and B₅ (Briles' classification); and B_A, B_C and B₁ (unpublished data).

Detection of MHAs on cell surface: To examine the reactivities of the cells with 13 specific antisera, the membrane immunofluorescence test (IFT) was performed by the indirect method as described previously [32] using fluorescein isothiocyanate-conjugated anti-chicken IgG goat serum. The percent of positive cells was established by counting a minimum of 200 cells. Reactivity below 10% is considered nonspecific. The titers were expressed as the reciprocal of the highest dilutions of sera at which the percentage of fluorescence positive cells was more than 10.

RESULTS

MHAs on various MD cell lines: Initially, the reactivity of six cell lines with 13 antisera to MHAs of the chicken B complex were examined by IFT (Table 1). The JP1 and JP2 cells reacted only with an antiserum specific to B_A. The HP1 cells reacted with antisera specific to B_A, B_M, B_N and B_O, while the HP2 cells reacted with antisera specific to B_A, B_G, B_M and B_O. The RP1 cells reacted with antisera specific to B_M and B_O. The MSB1-E cells reacted with anti-B_A and anti-B_K sera. These results indicated that these MD cell lines differed in their expression of MHAs.

MHAs on four sublines derived from MSB1: To study the antigenic diversity of MHAs on MD cell lines in detail, the reactivities of four sublines derived from MSB1 were examined with the 13 specific anti-B sera by IFT. Table 2 shows that there were some differences among the reactivities even in the four sublines. The MSB1-0, MSB1-E and MSB1-33C reacted with anti-B_K serum and also with two or three antisera specific to either B_A, B_D or

B_E. MSB1-41C cells reacted with anti-B_D serum and anti-B_M serum. These cells also reacted with various other antisera, but the percentage of the fluorescence positive cells was low and the reactivities of the cells were weak. These results indicate that MSB1 cell line consisted of cells having different MHAs on their surface, even though the cells had been cloned three times before we received them [1].

MHAs on 18 clones derived from MSB1-0: To confirm the changes of MHA expression in subpopulations of MSB1 cells, we attempted to obtain clones with distinct MHA from MSB1-0, which had been cultured for less than one year. Eighteen clones were obtained from MSB1-0 subline by cloning them three times in soft agar. The reactivity of these clones with specific antisera in the IFT is shown in Table 3. Eleven clones reacted only with both anti-B_E and B_K sera. Besides the reactivities with these two antisera, three clones reacted with anti-B_M serum, one clone with anti-B_A serum and one clone with anti-B_D serum. The other two clones reacted with

Table 1. Reactivities of 13 specific antisera to histocompatibility antigens of chicken B system against cell lines derived from Marek's disease lymphomas

Antiserum specific to B complex alloantigen	Agglutination titer (2 units) to homologous red blood cells	Titer ^{a)}					
		JP1	JP2	HP1	HP2	RP1	MSB1-E
A	4	>32 (15) ^{b)}	>32 (23)	16 (55)	4 (32)	—	32 (41)
B	4	— ^{c)}	—	—	—	—	—
C	4	—	—	—	—	—	—
D	2	—	—	—	—	—	—
E	4	—	—	—	—	—	—
G	2	—	—	—	32 (92)	—	—
I	2	—	—	—	—	—	—
K	4	—	—	—	—	—	4 (38)
L	2	—	—	—	—	—	—
M	2	—	—	8 (58)	4 (54)	2 (30)	—
N	4	—	—	4 (45)	—	—	—
O	2	—	—	16 (96)	8 (39)	2 (25)	—
T	2	—	—	—	—	—	—

a) Titers were determined by membrane immunofluorescence test.

b) Numbers in parenthesis are the percent positive cells at the serum dilution equal to the agglutination titer.

c) Negative.

Table 2. Reactivities of 13 specific antisera to histocompatibility antigens of chicken B system with cells in sublines derived from MSB1 cell line as determined by membrane immunofluorescence test^{a)}

Antiserum specific to B complex alloantigen	Agglutination titer (2 units) to homologous red blood cells	Fluorescence positive cells (%)			
		MSB1-O	MSB1-E	MSB1-33C	MSB1-41C
A	2	4	50	39	4
B	4	4	— ^{b)}	—	—
C	2	—	—	—	—
D	2	—	2	58	20
E	2	75	—	5	—
G	2	—	—	—	—
I	2	—	—	—	—
K	2	70	70	59	—
L	2	5	—	2	—
M	2	2	—	2	10
N	3	5	—	—	—
O	2	3	—	—	—
T	2	—	—	—	—

a) The membrane immunofluorescence tests were performed by using 13 antisera at the serum dilution equal to the agglutination titer.

b) Negative.

Table 3. Reactivities of 13 specific antisera to histocompatibility antigens of chicken B system with cells in clones derived from MSB1-O as determined by membrane immunofluorescence test^{a)}

Clone No.	Fluorescence positive cells (%)				
	anti-BA serum	anti-BD serum	anti-BE serum	anti-BK serum	anti-BM serum
1	— ^{b)}	—	77	92	14
2	41	—	75	92	—
3	—	—	73	72	—
4	—	—	65	73	—
5	—	—	72	73	—
6	—	—	—	68	30
7	—	—	55	65	—
8	—	—	65	69	—
9	—	—	70	86	—
10	—	—	53	90	—
11	—	—	46	62	18
12	—	—	—	89	—
13	—	—	40	73	—
14	—	—	55	78	—
15	—	16	61	63	—
16	—	—	48	67	17
17	—	—	66	70	—
18	—	—	59	60	—

a) The membrane immunofluorescence tests were performed by using 13 antisera at the serum dilution equal to the agglutination titer indicated in Table 2. None of the cells reacted with the other 8 antisera.

b) Negative.

both anti-B_K and anti-B_M sera or anti-B_K serum alone. These results indicate that MSB1-0 gave rise to at least 6 populations with differing MHA expression.

Changes in the MHA expression on MD cell lines: To study the possibility of a change in the expression of the MHA in long-term culture, we cultured MSB1-E, MSB1-0, MSB1-41C, clones No. 2, No. 6, No. 12 and RP1 cells for more than six months, and compared the reactivity of these cells with the 13 antisera by IFT (Table 4). After 11 months' culture, MSB1-0 cells became positive with anti-B_M serum. The reactivity of MSB1-E cells with anti-B_A and anti-B_K sera was almost the same as 18 months before, but they acquired a reactivity with anti-B_D and anti-B_L sera. MSB1-41C cells still did not react with anti-B_K serum after 14 months' culture and/or *in vivo* passage (data are not shown). The reactivities of clones No. 2 and No. 6 cells with antisera specific to B_A, B_E, B_K or B_M decreased after 7 months' culture. The cells in clone No. 12

became positive with anti-B_E serum but the intensity of the IFT reaction was weak. RP1 cells became positive with anti-B_A and anti-B_I sera after 18 months' culture. These results indicate that changes in MHA expression and in general a reduction in reactivity with anti-sera had occurred during long-term culture, and that fluctuation of MHAs is perhaps demonstrated in the same clone when it is investigated after short intervals.

DISCUSSION

Recently, attempts have been made to compare the MHC haplotypes present in chicken flocks in different parts of Europe and North America, and to establish a uniform nomenclature for chicken MHC genes and their products [5]. However, comparisons of the chicken flocks in those countries and in Japan have not yet been made. Furthermore, B antigens expressed on MD lymphomas have not been characterized in terms of the proposed nomenclature. We have therefore adopted the system of nomenclature used in Japan

Table 4. Reactivities of monospecific antisera to histocompatibility antigens of chicken B system with Marek's disease lymphoblastoid cells at two different times by membrane immunofluorescence test^{a)}.

Cell lines	Experiment time	Fluorescence positive cells (%)									
		anti-B _A serum	anti-B _B serum	anti-B _D serum	anti-B _E serum	anti-B _I serum	anti-B _K serum	anti-B _L serum	anti-B _M serum	anti-B _N serum	anti-B _O serum
MSB1-0	1981.11	4	4	— ^{b)}	75	—	70	5	2	5	3
	1982.10	2	—	—	26	—	66	—	39	—	—
MSB1-E	1981. 7	50	—	2	—	—	70	—	—	—	—
	1983. 1	36	—	16	—	—	95	11	—	—	—
MSB1-41C	1981.11	—	—	20	—	—	—	—	10	—	—
	1983. 1	—	—	—	20	NT ^{c)}	—	NT	—	—	NT
Clone No. 2	1982. 3	41	—	—	75	—	92	—	—	—	—
	1982.10	6	—	—	41	—	23	—	—	—	—
Clone No. 6	1982. 3	—	—	—	—	—	68	—	30	—	—
	1982.10	—	—	—	—	—	54	—	16	—	—
Clone No. 12	1982. 3	—	—	—	—	—	89	—	—	—	—
	1982.10	—	—	—	63	—	83	—	—	—	—
RP1	1981. 7	—	—	—	—	—	—	—	30	—	25
	1983. 1	—	19	—	—	33	—	—	32	—	51

a) The membrane immunofluorescence tests were performed by using 13 antisera at the serum dilution equal to the agglutination titer indicated in Table 2. None of the cells reacted with the other 3 antisera.

b) Negative.

c) Not tested.

Table 5. The MHA expression on Marek's disease lymphoma cells

Name of cell line	MHAs	Method for detection	Adopted nomenclature	Reference
MSB1	B ₁₉ , B ₂₁	IFT ^{a)}	Schmid	6
	B ₁₃	unknown	Hy-Line	19
	B _A , B _D , B _K	CT ^{b)}	Okada and McDermid	23
	B _K	LT ^{c)}	Okada and McDermid	23
RP1	B ₁₃	unknown	Briles	19
	B ₁	AT ^{d)}	Hy-Line	18
	B ₁₉	IFT	Schmid	6
	B _G , B _I	LT	Okada and McDermid	23
	B _G	CT	Okada and McDermid	23
HP1	B ₈	IFT	Schmid	6
HP2	B ₈	IFT	Schmid	6
JP1	B _A , B _K	IFT	Okada and McDermid	35
JP2	B _A , B _K	IFT	Okada and McDermid	35

a) Membrane immunofluorescence test.

b) Cytotoxicity test.

c) Lymphoagglutination test.

d) Absorption test.

[22].

Many cell lines have been established from MD lymphomas of chickens of highly inbred flocks [7, 8], but most of the early established cell lines were derived from outbred birds of unknown genotypes. The MHAs expressed on the cell lines used in the present studies have previously been investigated and the results are summarized in Table 5. Although it is difficult to compare directly the present results with those of the previous reports, it seems obvious that each cell line expresses different MHAs. Comparing the results of Table 1 with those of our previous studies of MHA on MSB1 or RP1 cells, some antigenic differences of MHA were noticed even within the same cell line [23]. With regard to antigenic differences between cell lines, JP1 and JP2 cells, derived from MD lymphomas of inbred chickens with B^AB^K blood groups, carried B_A and B_K alloantigens on their surface at the time of establishment [35]. However, the cells lost their reactivity with anti-B_K serum but not with anti-B_A serum during two years of cultivation (Table 1). These results led us to speculate that MD lymphoma cell lines might consist of cells carrying

different MHAs and that antigenic changes might occur during *in vitro* cultivation.

The existence of subpopulations in the cultured cells of mouse and human leukemia cell lines with differing expression of MHAs has been reported [11, 16]. However, differing expression of MHAs on chicken cell lines has not been reported. The present studies revealed that MHAs among four sublines and 18 clones derived from MSB1 cells are not constant. Most of these cells reacted constantly and strongly with anti-B_K serum but the degree of the reactivities with other sera was various. Since MSB1 cell line was derived from MD lymphoma of outbred chicken, the cells might have a possibility to react with various anti-B sera ever since the time of establishment.

Changes in the expression of HLA antigen on human lymphocytes after 5–36 months' culture have been reported [12]. Moreover, new or modified HLA antigen was detectable on normal human lymphocytes after culture for 3–4 days [20]. Considering these results, it is possible to speculate that *in vitro* changes might occur in the expression of MHAs even on chicken lymphoblastoid cells. Comparing

the antigenic properties of the sublines and clones derived from MSB1 cells before and after cultivation for more than six months, it is clear that some cells acquired or lost their reactivity with certain anti-B serum.

Several explanations have been offered for these changes in the expression of alien MHAs on tumor cell surfaces [27]. The first hypothesis is that the unexpected MHAs detected by serological means are actually not alien but represent a mere amplification of previously unknown public specificities present in minute, undetectable amounts, or even completely hidden on normal cells. The second is that a mutation of genes may give rise to products cross-reacting with normal MHAs of allogeneic haplotypes. The third is the derepression of silent genes present in the tumor cells. The exact mechanism for the changes in MHA expression on MSB1 cells and other MD lymphoma cell lines is not known. However, the frequency of the antigenic changes in MSB1 cells, which was derived from outbred chicken, was rather high and the changed antigenicities seemed to be unstable during cultivation *in vitro*. It is of interest to point out that such antigenic diversity of MHA was not observed in JP1 and JP2 cells, derived from inbred chickens fixed for MHA. These results might suggest that the different expression of the MHA in MSB1 sublines or clones is possibly caused by the derepression of silent genes in the tumor cells. The amplification of previously unknown normal antigens could occur in both inbred and outbred chicken cells. The mutation of genes might not occur so frequent and its phenotype might not be so unstable.

MSB1-41C is the first transplantable subline obtained from the originally non-transplantable MSB1 cell line [21]. RP1 cell line was derived from the JMV transplant [25]. In contrast to the non-transplantable MD cell lines (MSB1-E, MSB1-0, MSB1-33C, HP1, HP2, JP1 and JP2) the transplantable cells reacted weakly to antisera specific to B com-

plex antigens. It is reported that Ia-like antigen of the chicken is closely linked with the MHC [13], and is present on all MD cell lines tested [30]. However, this antigen appeared to be present at a lower concentration on the RP1 than on the other MD cell lines [30], and a number of the cell lines positive for Ia failed to transplant [14, 30]. Furthermore, progression or regression of the transplant tumors was independent of genetic susceptibility of chickens to MD [14]. Therefore our results might indicate that transplantability is related to the degree of MHA expression of the donor cells.

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要 約

マレック病リンパ腫由来株化細胞における主要組織適合抗原の発現: 東原朋子・見上 彪・小沼 操・伊澤久夫・松田治男¹⁾・岡田育穂²⁾ (北海道大学獣医学部家畜伝染病学講座, ¹⁾広島大学生物生産学部家畜衛生学講座・²⁾畜産科学講座)——マレック病リンパ腫 由来の6種の細胞 (MDCC-JP1, JP2, HP1, HP2, RP1, MSB1) における主要組織適合抗原 (MHA) の発現に関して, 間接膜蛍光抗体法で検索を行なった。これらの細胞では, さまざまの MHA の発現が認められたが, MHA 発現の差異は, MSB1 細胞由来の4種の亜株および18種のクローン間でも認められ, MSB1 細胞が異なる MHA を発現する細胞群の集団からなる可能性が示唆された。さらに, これらの株化細胞のうち数種について, 6ヶ月以上培養を続けた後の MHA 発現を調べたところ, 抗原性の変化あるいは低下が認められた。以上の結果から, 試験管内における長期培養によって株化細胞の MHA が変化する可能性が示唆された。