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SHORT COMMUNICATION

DEMONSTRATION OF A MAREK'S DISEASE TUMOR-ASSOCIATED SURFACE ANTIGEN (MATSA) ON SIX CELL LINES DERIVED FROM MAREK'S DISEASE LYMPHOMAS

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Studies on a Marek's disease (MD) tumor cell-associated antigen have been greatly facilitated by the successful establishment of lymphoblastoid cell lines from MD lymphomas, e.g., the MOB-1 (Akiyama et al., 1973), MSB-1 (Akiyama and Kato, 1974), MOB-2 (Ikuta et al., 1976), and the HPRS Line 1 and HPRS Line 2 lines (Powell et al., 1974). The MOB-3 line was recently established from an ovarian tumor of a SPF chicken inoculated with MSB-1 cells using the method described by Akiyama and Kato (1974). The MOB-3 line cells are similar to the MSB-1 line cells in various biological characteristics, associated with MD virus (MDV) and being free from gs antigen of avian leukosis virus (ALV). However, karyological study revealed that the MOB-3 line did not show any recognizable chromosomal aberration (Takagi et al., unpublished), while the MSB-1 line showed specific chromosomal aberration (Akiyama and Kato, 1974). Powell et al. (1974) reported that cells of the HPRS Line 1 and of MD lymphomas were reactive by membrane immunofluorescence with serum from a rabbit immunized with MD lymphoma cells and suggested that these cells carried a tumor-specific antigen. With serum from rabbits immunized with MSB-1 cells or from chickens immunized with JMV cells, Witter et al. (1975) also demonstrated surface antigenic markers on three classes of MD tumor cells, i.e., MSB-1 cells, JMV lymphoblastic leukemia cells and MD lymphoma cells by indirect membrane immunofluorescent staining. They provisionally designated this antigen as a Marek's disease tumor-associated surface antigen (MATSA).

In this work, using sera from rabbits and chickens immunized with MSB-1 cells, we tested for MATSA on all the six MD lymphoblastoid cell lines described above and MD ovarian lymphoma cells, by the immunofluorescent technique. Specific antisera against MSB-1 cells were prepared in rabbits and chickens immunized with MSB-1 cells. Pairs of rabbits were given three injections each of 2×10^8 MSB-1 cells. For the first injection, the cells were mixed with incomplete adjuvant consisting of 4 parts of *n*-hexadecane and 1 part of glycerol monooleate (Crowle, 1973) and injected subcutaneously. For the following 2 booster injections 4 and 6 weeks later, the cells were injected intravenously. On the other hand, five 6- to 8-week-old

chickens were given three injections each of 2×10^8 MSB-1 cells which had been fixed with McLean-fixative (McLean and Nakane, 1974) to prevent infection of the chickens with MDV derived from the line cells. In two of five chickens, the cells were mixed with incomplete adjuvant for the first and second injections, and injected intramuscularly with a one-week interval between injections. For the last injection, the cells were injected intravenously without adjuvant 3 weeks later. In three of five chickens, the cells were injected intravenously without adjuvant at 2-week intervals. Anti-MSB-1 sera from rabbits and chickens were collected 7 days after the last injection, and inactivated by heating at 56 C for 30 min. Rabbit anti-MSB-1 sera were absorbed extensively with a mixture of bursal, thymic and splenic cells from normal chickens until reactivity against normal chicken lymphocytes by membrane immunofluorescence had been completely lost. The sera were provisionally named anti-MSB-1/R1 and R2, respectively. The two chicken anti-MSB-1 sera from chickens immunized with cells with adjuvant were named anti-MSB-1/C1 and C2, respectively, and the three sera from chickens immunized with cells without adjuvant were named anti-MSB-1/C3, C4 and C5, respectively. The sera from chickens infected with the MDV C2 and herpesvirus of turkeys (HVT) 01 strains, respectively, were used to demonstrate viral intracellular antigen (VA) and membrane antigen (MA). The sera were also obtained from MD lymphoma-bearing chickens, normal SPF chickens and normal rabbits. The avian lymphoma cells examined were the six MD lymphoblastoid line cells described above, 1104-B line cells (Hihara et al., 1974) from avian bursal lymphoma, induced by avian leukosis virus, and MD lymphoma cells. HPRS Line 1 and Line 2 line cells, and 1104-B line cells were kindly provided by Dr. P. C. Powell of Houghton Poultry Research Station, England, and by Dr. H. Hihara of the National Institute of Animal Health, Japan, respectively. MDV (C2 strain) -infected and non-infected chick embryo fibroblasts (CEF), and bursal, thymic and splenic cells from 1- to 2-week-old SPF chickens were also examined.

MATSA was examined by the indirect membrane immunofluorescent technique as described by Witter et al. (1975). MDV-induced MA (Chen and Purchase, 1970) and MD VA were also examined by the immunofluorescent technique as described by Naito et al. (1970).

The results are shown in Table 1. Anti-MSB-1/R1 serum reacted with a high proportion of all six MD lymphoblastoid line cells and with up to 27.0% of the MD ovarian lymphoma cells but not with normal lymphocytes, normal CEF, MDV-infected CEF or 1104-B line cells. Anti-MSB-1/R2 serum and anti-MSB-1/C1 serum showed similar reactions with these cells. The pooled sera of chickens infected with MDV, which possessed VA and MA reactivity, rarely stained MD ovarian tumor cells. The 4 MD lymphoblastoid cell lines (MSB-1, MOB-1, MOB-2, MOB-3) contained a few per cent or less of VA and MA positive cells, and the HPRS Line 1 and Line 2 rarely contained these cells. Thus most of cells constituting these lines seem nonproducers. These results may indicate that MATSA is unrelated to known MDV-induced antigens demonstrated in the productive cycle of infection. These MD lymphoblastoid line cells carry T cell determinants (Matsuda et al., 1976; Powell et al., 1974; Nazerian and Sharma, 1975). Lack of MATSA in any normal lymphocytes including normal T lymphocytes indicates the irrelevance of MATSA to T cell determinants of these line cells. MOB-2 line cells were found to be dually infected with ALV and MDV (Ikuta et al., 1976). Superinfection with ALV, however, did not have any recognizable influence on the antigenicity of MATSA. Normal CEF taken from 10 day-old chick embryos did not have any recognizable MATSA. Our preliminary experiments revealed that embryonic thymus cells with T cell determinants in about 90% pro-

TABLE 1. *Membrane immunofluorescence on various cells stainea with sera from rabbits and a chicken immunized with MSB-1 cells*

Cells		% MATSA positive cells[a]			% Viral antigen positive cells[a]	
					VA	MA
		Anti-MSB-1/R1[b] (1 : 25)[d]	Anti-MSB-1/R2[b] (1 : 20)[d]	Anti-MSB-1/C1[c] (1 : 32)[d]	Anti-MDV (1 : 10)[d]	
MSB-1		88.2	73.5	91.3	2.0	1.0
MOB-1		91.2	54.7	89.7	0.4	0.5
MOB-2		86.2	71.8	88.7	0.2	0.1
MOB-3		90.9	81.2	94.5	1.2	0.6
HPRS Line 1		94.8	NT[e]	96.5	0.0	0.0
HPRS Line 2		92.4	NT	94.6	0.0	0.0
MD ovarian lymphoma	1	23.2	NT	14.4	NT	NT
	2	14.6	NT	9.1	NT	NT
	3	27.0	NT	17.4	NT	NT
	4	NT	21.2	NT	0.0	0.0
	5	NT	13.3	NT	0.0	0.0
	6	NT	11.7	NT	0.0	0.0
1104-B		0.0	0.0	0.2	0.0	0.0
MDV-inf. CEF		0.0	0.0	0.0	+	+
Normal CEF		0.0	0.0	0.0	−	−
Normal thymus		0.0	0.0	0.0	0.0	0.0
Normal bursa		0.0	0.0	0.3	0.0	0.0
Normal spleen		0.0	0.0	0.2	0.0	0.0

[a] 300 to 400 single cells were scored.

[b] Rabbit anti-MSB-1/R1 and anti-MSB-1/R2 sera were reactive against more than 50% of the MSB-1 cells up to 1 : 128 and 1 : 32 dilution, respectively.

[c] Chicken anti-MSB-1/C1 serum, was reactive against more than 50% of the MSB-1 cells up to 1 : 128 dilution.

[d] Antisera at these dilutions were applied.

[e] Not tested.

portions, taken from 17–19 day-old chick embryos contained few cells reactive to MATSA antibody (Matsuda et al., unpublished). These results indicate that most of embryonic T cells, if not all, do not contain MATSA. The 1104-B line cells transformed by ALV subgroup A were free from any recognizable MATSA. Thus MATSA is not necessarily associated with transformation of avian lymphocytes with other oncogenic virus. These findings indicate that MATSA was specifically associated with most cells of all six MD lymphoblastoid cell lines, and with MD lymphoma cells in proportions varying from 9.1% to 27.0%. Similar results were obtained using the peroxidase-labeled antibody technique of Nakane and Kawaoi (1974) (Matsuda et al., in preparation).

The titers of MATSA antibody in various chicken sera were measured by the indirect immunofluorescence technique. As shown in Table 2, sera from chickens infected with MDV or HVT and sera from chicks with MD lymphoma also did not react against MATSA.

TABLE 2. *Titers of MATSA antibody in various chicken sera measured by indirect immunofluorescence*

Serum	MATSA titer against MSB-1 cells	Serum	MATSA titer against MSB-1 cells
Anti-MSB-1/C1[a]	128	Anti-MDV[c]	<4
Anti-MSB-1/C2[a]	128	Anti-HVT[d]	<4
Anti-MSB-1/C3[b]	<4	MD tumor-bearing chickens 1	<4
Anti-MSB-1/C4[b]	<4	MD tumor-bearing chickens 2	<4
Anti-MSB-1/C5[b]	<4	MD tumor-bearing chickens 3	<4
MD free chickens 1	<4	MD tumor-bearing chickens 4	<4
MD free chickens 2	<4	MD tumor-bearing chickens 5	<4

[a] These sera were obtained form chickens immunized with MSB-1 cells with adjuvant.
[b] These sera were obtained from chickens immunized with MSB-1 cells without adjuvant.
[c] The serum was a pooled sample of sera from MDV-infected chickens.
[d] The serum was a pooled sample of sera from HVT-infected chickens.

The results also indicate that MATSA is distinct from known virus-associated antigens. Anti-MSB-1/C1 and anti-MSB-1/C2 sera, from chickens immunized with MSB-1 cells with adjuvant both reacted against MATSA on MSB-1 cells at a high dilution of $\times 128$. However, the sera, anti-MSB-1/C3, anti-MSB-1/C4 and anti-MSB-1/C5, from chickens immunized with MSB-1 cells without adjuvant, did not react against MATSA.

There are 2 possible explanations for why none of the five sera obtained from chickens bearing MD lymphoma reacted against MATSA. One is that, since we failed to obtain MATSA antibody in sera (anti-MSB-1/C3–5) from chickens immunized with MSB-1 cells without adjuvant, the antigenicity of MATSA may be too low to induce demonstrable humoral antibody in chickens. The other is that chickens with MD lymphoma may not be able to respond well to MATSA due to some disturbance of function of the immunosurveillance system. In fact, depression of the antibody response has been demonstrated in chickens infected with MDV (Kleven et al., 1972). Furthermore, Theis et al. (1975) have shown marked depression of responsiveness to the T cell mitogen phytohemagglutinin in chickens with MD visceral lymphomas. If MATSA antibody formation is mediated through thymus function as well as bursal function, the low responsiveness to MATSA in chickens with MD lymphoma is understandable.

Conclusively with sera of rabbits and chickens immunized with MSB-1 cells, we could specifically demonstrate the MATSA reported by Witter et al. (1975) on most cells of all six MD lymphoblastoid cell lines and on a certain proportion of MD lymphoma cells.

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