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SHORT COMMUNICATION

DEMONSTRATION OF CELLS WITH MAREK'S DISEASE TUMOR-ASSOCIATED SURFACE ANTIGEN IN CHICKS INFECTED WITH HERPESVIRUS OF TURKEY, O1 STRAIN[1]

NORITOSHI KITAMOTO, KAZUYOSHI IKUTA, and SHIRO KATO

Department of Pathology, Research Institute for Microbial Diseases, Osaka University, Yamada-kami, Suita, Osaka 565, Japan

KAZUMASA WATAKI

Hokuwa Livestock Hygiene Service Center, Nara 639–11, Japan

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The presence of Marek's disease tumor-associated surface antigen (MATSA) was demonstrated by the direct and indirect membrane immunofluorescent tests, in chicks inoculated 7–10 days earlier with herpesvirus of turkeys (HVT), O1 strain. In in vitro cultures of spleen lymphocytes and ovaries obtained from these chicks, MATSA-positive cells were also detected after 1–7 days cultivation. A possible mechanism of protection by HVT vaccine against Marek's disease is proposed.

Marek's disease (MD) is a naturally occurring malignant lymphoma of chickens caused by a herpesvirus named MD virus (MDV). It is the only known malignant tumor that can be prevented by vaccination. For vaccination, herpesvirus of turkeys (HVT) is now extensively used as live vaccine virus, but the mechanism of its protective effect is poorly understood. Only a few workers (Sharma et al., 1978; Kitamoto et al., 1979) have reported on the cell-mediated cytotoxic response of lymphocytes obtained from HVT-infected chickens against MD lymphoma cell line, MSB-1 (Akiyama and Kato, 1974). Because MSB-1 cells contain MATSA, it was postulated that the immune response detected in the vaccinated chickens may be directed against this antigen. Although HVT is generally considered not to be pathogenic in chickens, Witter et al. (1976) found that chickens infected with HVT developed transitory lymphoproliferative lesions during the early stage of infection and postulated that the lymphoblastic cells in HVT lesions may contain MATSA. Recently, Powell and Rennie (1978) and Schat and Calnek (1978) detected the presence of MATSA by the indirect membrane immunofluorescent (IF) test on lymphocytes from chickens vaccinated with HVT, FC 126, which was isolated by Witter et

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al. (1970).

In this work, using both indirect and direct IF tests, we demonstrated the early appearance of MATSA in chicks infected with HVT, O1 strain, which was isolated in our laboratory (Ono et al., 1974). We also found MATSA-positive cells in in vitro cultures of lymphocytes obtained from HVT-infected chicks.

The chickens used were from a specific pathogen-free (SPF) flock of SPAFAS (SPAFAS Inc., Norwich, Connecticut U.S.A. Eggs were kindly provided by Bio Pharmaceuticals Inc., Tokyo). The eggs were confirmed to be free from antibodies to HVT and MDV, and the chicks were kept in isolation from the day of hatching throughout the experiment. The O1 strain of HVT (Ono et al., 1974) was used. The virus was propagated in chick embryo fibroblasts (CEF) and infected cells were stored at −70 C until use. One-day-old chicks were inoculated intra-abdominally with 5,000 plaque-forming units (PFU) of HVT/0.2 ml/chick. Uninoculated hatchmates and, in some cases, CEF-inoculated hatchmates were used as controls. On appropriate days, chicks from each group were killed by cardiac puncture. The tissues, e.g., spleen, ovary, thymus and bursa were chopped into small pieces with scissors and suspended in PBS. Single cell suspensions were prepared by passing the suspensions through a stainless-steel wire sieve. Spleen lymphocytes (SPL) and peripheral lymphocytes (PBL) were separated by layering them on a Lymphoprep (Nyegaard & Co. Oslo, Norway), and centrifuging them at 1,500 rpm for 20 min. Then they were washed three times with PBS. Suspensions of cells from other tissues were also washed three times with PBS. The resulting packed cells were examined for the presence of MATSA-positive cells by the indirect and direct IF tests.

Rabbit anti-MSB-1 cell serum, used in the indirect IF test, was prepared as described by Matsuda et al. (1976). Specific anti-MATSA serum was prepared by extensive absorption with chicken erythrocytes, bursa, thymus and spleen cells until all reactivity against normal chicken lymphocytes had been removed. The serum was also absorbed with 1104-B line cells (Hihara et al., 1974), whcih were derived from an avian bursal lymphoma induced by avian leukosis virus (This cell line was kindly provided by Dr. H. Hihara of the National Institute of Animal Health, Tokyo), and used at a dilution of 1: 16. Goat anti-rabbit globulin serum was labeled with fluorescein-isothiocyanate (FITC) as described by Natio et al. (1969). Chicken anti-MSB-1 cell serum, used in the indirect and direct IF tests, was prepared as described by Matsuda et al. (1976). This serum was absorbed with 1104-B cells. Rabbit anti-chicken globulin serum, used in the indirect IF test, and chicken anti-MATSA serum, used in the direct IF test, were labeled with FITC as described by Naito et al. (1969). Chicken anti-MATSA serum (1: 16) and FITC-conjugated chicken anti-MATSA serum (1: 4) did not stain normal chicken lymphocytes or 1104-B cells. At least 5,000 cells were counted in IF tests.

As shown in Table 1, MATSA-positive cells were demonstrated in chicks inoculated with HVT 7–10 days previously. However, at this time, MATSA-postive cells were not detected in all the chicks inoculated with HVT, and no MATSA-positive cells were observed before or after this time in HVT-infected chicks, or at any time in control chicks. Moreover, no MATSA-positive cells were found among the PBL.

The percentage of MATSA-positive cells among the SPL of chicks inoculated with HVT was examined by the indirect IF test using rabbit or chicken anti-MATSA serum ,and by the direct IF test using FITC-conjugated chicken anti-MATSA serum (Table 2). The percentage of these cells in vaccinated chicks was very low, the maximum being 0.2% as detected by the direct IF test using FITC-conjugated chicken anti-MATSA serum. Moreover only a few MATSA-positive cells were seen in other tissues, such as the ovary, thymus and bursa (0.1–0.2%). The fluorescence of the cells was restricted to the membrane and was seen as a

TABLE 1. *Incidence of chicks with MATSA-positive cells detected by indirect and direct IF tests among those inoculated with HVT*

IF test	Cells	Days after inoculation[a] (No. positive/No. tested)[b]												Control chicks
		5	6	7	8	9	10	11	14	21	28	35	42	
Indirect[c]	SPL	0/5	0/5	9/28	6/18	0/3	1/7	—	0/4	0/2	0/4	0/4	0/4	0/35
	PBL	0/2	0/1	0/20	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/2	0/2	0/21
	Ovary	—[e]	—	6/12	2/12	1/3	0/3	—	—	—	—	—	—	0/7
Direct[d]	SPL	0/6	—	6/13	3/5	—	0/4	—	0/2	0/2	0/2	0/2	0/2	0/16
	PBL	0/3	—	0/8	0/3	—	0/2	—	0/2	—	—	0/2	0/2	0/16
	Ovary	0/2	—	1/5	1/3	—	0/2	—	0/2	—	—	—	—	0/5
	Thymus	0/2	—	2/3	2/5	—	0/2	—	—	—	—	—	—	0/4
	Bursa	0/2	—	0/3	1/5	—	—	—	—	—	—	—	—	0/5

[a] Chicks were inoculated with HVT O1 strain (5,000 PFU/0.2 ml/chick).
[b] Numerator ; No. of chicks with MATSA-positive cells. Denominator ; No. of chicks tested.
[c] Rabbit anti-MATSA serum was used at a dilution of 1 : 16.
[d] FITC-conjugated chicken anti-MATSA serum was used at a dilution of 1 : 4.
[e] Not tested.

TABLE 2. *Percent of MATSA-positive cells in SPL of chicks inoculated with HVT*

Days after inoculation[a]	No. of chicks	IF test[b]		
		Indirect		Direct[e]
		Rabbit[c]	Chicken[d]	
7	1	0	—[f]	0
	2	0	—	<0.1
	3	0	0	0
	4	—	<0.1	0.1
	5	—	0	0
	6	<0.1	<0.1	0.2
	7	0	<0.1	0
	8	0	0	0
	9	0	0	0
	10	0	0	0.2
8	1	0	<0.1	<0.1
	2	<0.1	<0.1	<0.1
	3	0	0	0

[a] Chicks were inoculated with HVT, O1 strain (5,000 PFU/0.2 ml/chick).
[b] At least 5,000 cells were counted.
[c] Rabbit anti-MATSA serum was used at a dilution of 1 : 16.
[d] Chicken anti-MATSA serum was used at a dilution of 1 : 16.
[e] FITC-conjugated chicken anti-MATSA serum was used at a dilution of 1 : 4.
[f] Not tested.

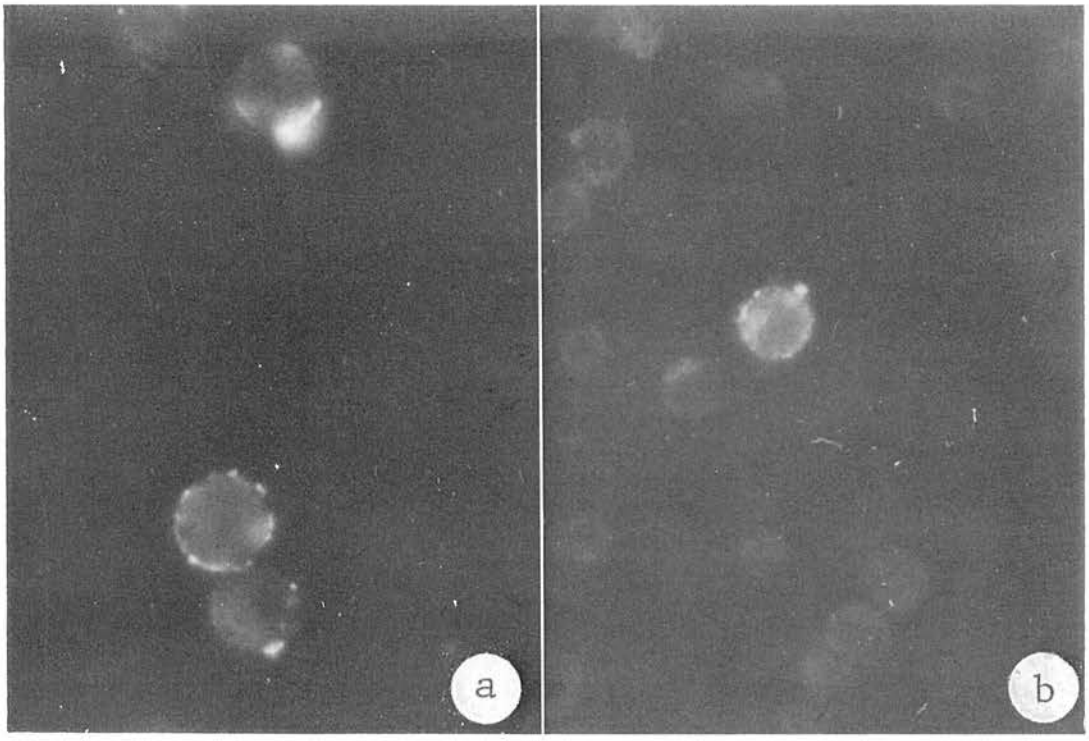

FIGURE 1. The presence of MATSA was demonstrated in HVT-infected chick, by the direct membrane immunofluorescent test using FITC-conjugated chicken anti-MATSA serum. (a) MSB-1 cells as positive control, (b) SPL obtained from HVT-infected chick at 7 days after infection.

ring or as patches (Fig. 1).

Next we examined whether MATSA-positive cells could be detected in in vitro cultures of lymphocytes obtained from HVT-infected chicks. Samples of SPL and ovary were obtained from HVT-infected chicks at 7 days after infection, as described above. The cells were suspended in growth medium (RPMI-1640 supplemented with 20% fetal bovine serum and 10% chicken serum), seeded into petri dishes (100×20 mm), and then cultured at 41 C in a humidified atmosphere of 5% CO_2 in air. At intervals during cultivation, samples of cells were collected, washed three times with PBS and examined for the presence of MATSA-positive cells by the direct IF test using FITC-conjugated chicken anti-MATSA serum. As shown in Table 3, MATSA-positive cells were found in in vitro cultures after 1–7 days cultivation. In some cases, MATSA-positive cells were not seen before cultivation, but became detectable during cultivation, and in other cases, the percentages of positive cells increased during cultivation.

This experiment demonstrated the early appearance of MATSA-positive cells in chicks inoculated with HVT, O1 strain. Our data essentially confirm the results of Schat and Calnek (1978). Powell and Rennie (1978) reported that MATSA-positive cells were first observed in chicks on day 10, and thereafter were found every day until the end of the experiment (66 days). This difference may be due to differences of experimental conditions, such as differences in the lines of chickens or strains of virus used. The time of appearance of MATSA-positive cells in our experiments is similar to that of the lymphoproliferative

TABLE 3. *Percent of MATSA-positive cells in in vitro culture of lymphocytes obtained from chicks inoculated with HVT*

HVT-	No. of chicks	Cells	Percent of MATSA-positive cells[a]										
			Days of cultivation[b]										
			0[c]	1	2	3	4	5	6	7	8	10	14
Infected	1	SPL	0.2	—[d]	0.8	0.6	—	<0.1	—	0	—	—	—
	2	SPL	<0.1	—	—	2.3	0.8	0.7	—	<0.1	—	—	—
	3	SPL	<0.1	—	—	0.7	—	0	—	0	—	—	—
	4	SPL	<0.1	<0.1	0	0	0	0	0	0	—	—	—
		Ovary	0	0	0	0.1	0	0	0	0	—	—	—
	5	SPL	0.1	0	0	0	0	0	0	0	—	—	—
		Ovary	<0.1	0	0	0	0	<0.1	0	0	—	—	—
	6	SPL	0	0	0	0	0	0	0	0	—	—	—
		Ovary	0	0	0	<0.1	0.4	0	0	0	—	—	—
	7	SPL	0	—	0	<0.1	—	0	—	0	0	0	0
	8	SPL	<0.1	—	—	0.1	—	0	—	0	—	—	—
	9-13	SPL	0	0	0	0	0	0	0	0	0	0	0
Un-infected	1	SPL	0	0	0	0	0	0	0	0	—	—	—
		Ovary	0	0	0	0	0	0	0	0	—	—	—
	2	SPL	0	0	0	0	0	0	0	0	—	—	—
		Ovary	0	0	0	0	0	0	0	0	—	—	—
	3-6	SPL	0	—	0	0	0	0	—	0	0	0	0

[a] At least 1,000-5,000 cells were counted by the direct IF test using FITC-conjugated chicken anti-MATSA serum.

[b] Cells obtained from chicks 7 days after inoculation were cultured.

[c] Cells before cultivation.

[d] Not tested.

lesions described by Witter et al. (1976). Expression of MATSA has been found to be closely associated with the MD-derived lymphoblastoid cell lines and MD lymphoma cells so far examined (Witter et al., 1975: Matsuda et al. 1976), but the tumor specificity of MATSA still requires further investigation. Sharma et al. (1978) and Kitamoto et al. (1979) reported that chickens inoculated with HVT developed a T-cell-mediated immune response to MATSA. Recently, a new lymphoblastoid cell line expressing MATSA, associated with HVT and MDV has been established from the spleen of an HVT-infected chicken (Kitamoto et al. in press). Thus conceivably upon HVT infection, some HVT-infected lymphocytes undergo transformation and express MATSA which stimulates cell-mediated and/or humoral immunity. This immunity could eliminate not only HVT-induced MATSA-positive cells but also MATSA-positive cells transformed by virulent MDV, which may infected after HVT vaccination.

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