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Citation	Biken journal : journal of Research Institute for Microbial Diseases. 1980, 23(1), p. 57-60
Version Type	VoR
URL	https://doi.org/10.18910/82540
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PRELIMINARY REPORT

DEMONSTRATION OF HETEROPHILE ANTIBODY IN CHICKEN ANTISERUM AGAINST MAREK'S DISEASE TUMOR-DERIVED CELL LINE, MSB-1

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(Received October 12, 1979)

Sheep red blood cells (SRBC) were agglutinated by all six chicken anti-MSB-1 sera examined, but not by sera of thirty specific pathogen-free chickens. The SRBC-agglutination titer was greatly reduced by absorption with SRBC, bovine red blood cells (BRBC) or guinea-pig kidney cells (GPKC). The dissociation of heterophile antibody and antibody to so-called Marek's disease tumor-associated surface antigen (MATSA) is discussed.

Marek's disease (MD) is an extensively studied lymphoproliferative disease of chickens caused by a herpesvirus named MD virus (MDV). MD is particularly useful as a model of oncogenic herpesviruses, such as Epstein-Barr virus (EBV), which is closely associated with Burkitt's lymphoma and nasopharyngeal carcinoma (Klein, 1973), and it is also considered to be the etiological agent of infectious mononucleosis (IM) (Henle et al., 1968). A Paul-Bunnell (PB) antibody is transiently induced in the sera of almost 100% of all IM patients (Hoagland, 1952; Leikowitz, 1953), and PB antigen has been demonstrated on the cell surface of EBV-transformed cell lines (Shope and Miller, 1973; Maeda et al., 1979). These observations prompted us to study whether heterophile antibody is present in chicken antiserum against MDCC-MSB-1

cells (Akiyama and Kato, 1974).

The only known tumor-associated cell surface antigen on MDV-transformed cells is MD tumor-associated surface antigen (MATSA) (Powell et al., 1974; Witter et al., 1975; Matsuda et al., 1976). However, the exact nature of this putative tumor antigen is not properly understood; MATSA has been proposed to be a modified T-cell antigen (Ross et al., 1977) or histocompatibility antigen (Bülow and Schmid, 1978), but both these possibilities are still doubtful (Murthy and Calnek, unpublished data). Preliminary tests were carried out to demonstrate the presence of heterophile antibody in chicken anti-MSB-1 sera which had been prepared as anti-MATSA sera.

MSB-1 cells were cultured in RPMI-1640 +10% fetal calf serum. Anti-MSB-1 sera

were prepared in chickens of more than 6-weeks-old which had been immunized with fixed MSB-1 cells with Freund's adjuvant, as described previously (Witter et al., 1975; Matsuda et al., 1976). Antisera to living MSB-1 cells, kindly supplied by Dr. T. Mikami, Hokkaido University, Japan, were also used. Agglutination titers were measured by microtitration in the presence of 1% fetal calf serum to exclude a nonspecific reaction. Titrations were performed by serial two-fold dilutions of antiserum in 25 μ l of phosphate-buffered saline (PBS), pH 7.4, and measurements were made at room temperature 3 hr after addition of 25 μ l of 0.5% sheep red blood cells (SRBC) in PBS containing 1% fetal calf serum. For titration of the staining titer to the MSB-1 cell surface, the indirect membrane immunofluorescence (MIF) test was performed as described previously (Matsuda et al., 1976). Titers are given as the reciprocal of the last dilution showing clearly demonstrable activity.

Table 1 shows the relation between the titer of the SRBC agglutination antibody and the staining titer to MSB-1 cells measured by the

TABLE 1. *Presence of heterophile antibody in chicken anti-MSB-1 sera.*

	Agglutination titer of SRBC	Titer to MSB-1 cells by MIF
1	64	320
2	4	40
3	4	160
Chicken anti-MSB-1 sera ^a	4	320
	5	40
	6	40
SPF chicken sera ^b	<2	<2

^a Lots 1 and 2, and lots 3-6 of anti-MSB-1 sera were prepared in chickens immunized with fixed MSB-1 cells and living MSB-1 cells, respectively.

^b All sera from twenty chickens of one-day-old, five chickens of 3-months-old, and five chickens of 6-months-old showed the same results.

indirect MIF test. Agglutinating activity was detected in all six chicken anti-MSB-1 sera examined, and the titers were between 4 and 64. However, the agglutination titers were not always correlated with the staining titers to MSB-1 cells measured by the indirect MIF test. These anti-MSB-1 sera reacted only slightly with the cell surface of normal chicken splenic lymphocytes or LSCC-1104B cells, which are an avian leukosis virus (ALV)-transformed cell line (Hihara et al., 1974). No antibody of SRBC-agglutination or antibody to MSB-1 cells by the indirect MIF test was detectable in the sera from thirty specific pathogen-free chickens of various ages. When chicken anti-MSB-1 serum was absorbed three times with SRBC, bovine red blood cells (BRBC) or guinea-pig kidney cells (GPKC), the reactivity with SRBC was completely eliminated (Table 2). For absorption of antiserum, living SRBC or BRBC, or boiled GPKC, which had been extensively washed with PBS, were suspended with 2 volumes of antiserum, and incubated for 30 min at 37°C. The heterophile antibody in chicken anti-MSB-1 serum seems fit for Hanganutziu-Deicher (HD) antibody which is absorbable with SRBC, BRBC or GPKC (Davidsohn, 1938). On the other hand, the reactivity with the cell surface of MSB-1 cells in the indirect MIF test, which has been defined as the titer of MATSA antibody, was considerably reduced by absorption with SRBC, BRBC or GPKC, but only slightly reduced by absorption with chicken red blood cells (CRBC).

TABLE 2. *Absorption test of anti-MSB-1 serum with various cells*

Absorbent cells	Agglutination titer of SRBC	Titer to MSB-1 cells by MIF
None	64	320
SRBC	<2	20
BRBC	<2	20
GPKC	<2	20
CRBC	32	160

These results indicate that chicken anti-MSB-1 serum may contain HD-like heterophile antibody. However, the antibody to MSB-1 cells staining by the indirect MIF test remained after absorption with SRBC, BRBC or GPKC, as shown in Table 2, and the heterophile antibody titers of chicken anti-MSB-1 sera did not always correlate with the titers of antibody staining MSB-1 cells by the indirect MIF test, as shown in Table 1. Thus the so-called MATSA proposed by Witter et al. (1975) seems to consist of at least two types of antigen, HD-like heterophile antigen and an other antigen.

HD antibody has been named "serum-sickness antibody" (Hanganutziu, 1924; Deicher, 1926). However, recent studies have shown that heterophile antibody of this type is present in sera of patients with various diseases who had never received any injection of a foreign species of serum (Kasukawa et al., 1976), and that this HD antigen has also been identified in extracts of various human malignant tissues (Nishimaki et al., 1979). Therefore, MSB-1 cells cultured in media with chicken serum were examined by the indirect MIF test to exclude the possibility that the HD-like heterophile antibody had developed in chicken anti-MSB-1 sera as a results of repeated exposure to the small amounts of fetal calf serum present in the cell culture

media and not removed by repeating washing. As shown in Table 3, the cell surface of MSB-1 cells cultured in media with chicken serum also gave a positive reaction in the indirect MIF test with chicken anti-MSB-1 serum. The intensity of the reaction was greater on MSB-1 cells cultured with chicken serum than on MSB-1 cells cultured with fetal calf serum. On the other hand, the cell surface of 1104B, an avian lymphoblastoid cell line transformed by ALV, reacted only slightly with this anti-MSB-1 serum, even when the cells were cultured with fetal calf serum in the same way as the MSB-1 cells. These results indicate that the HD-like heterophile antigen is expressed on the cell surface of MSB-1 cells, but not on that of 1104B cells, and that it is not due to fetal calf serum.

Further studies to characterize the HD-like heterophile antigen on MSB-1 cells are in progress.

ACKNOWLEDGMENTS

We are grateful to Drs. Y. Hinuma and T. Sairenji, Kumamoto University Medical School, Japan, for valuable discussion. This work was partly supported by a grant-in-aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

TABLE 3. Comparison of indirect MIF reactions with chicken anti-MSB-1 serum of cells cultured in medium with chicken serum and in medium with fetal calf serum

Cells	Cultivation with	Dilution of chicken anti-MSB-1 serum					
		20	40	80	160	320	640
MSB-1 cells	Fetal calf serum	>95 ^a	>95	>95	80	20	0
	Chicken serum	>95	>95	>95	>95	30	10
1104B cells	Fetal calf serum	<10	0	0	0	0	0
Normal chicken splenic lymphocytes		<10	0	0	0	0	0

^a Per cent of fluorescent cells.

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