BIKEN JOURNAL Vol. 16, 91-94, 1973

PRELIMINARY REPORT

CELL SURFACE ANTIGEN AND VIRAL ANTIGEN OF MAREK'S DISEASE VIRUS IN LYMPHOID CELLS OF THE SPLEEN FROM CHICKENS INOCULATED WITH VIRULENT STRAINS[1]

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(Received January 22, 1973)

Various states of dissociation of expressions of antigens in cells infected with Epstein-Barr virus (EBV) have been reported. Klein et al. (1966; 1968) reported EBV induced cell surface antigen (CSA) of Burkitt's lymphoma cells which are free from viral antigen (VA). This supports the idea of the intrinsic involvement of the EB viral genome in lymphoid cells. This idea was also strongly supported by results using the hybridization technique reported by Zur Hausen (1972). CSA's on cells infected with Marek's disease virus (MDV) and with herpesvirus of turkey (HVT) were reported by Chen and Purchase (1970) and Ishikawa et al. (1972), respectively. From experiments using antimetabolites, the latter authors suggested that CSA of HVT is a newly synthesized protein, coded by a parental viral genome. It is very difficult to do similar experiments with antimetabolites of CSA of MDV because of the cell-associated nature of infectivity of MDV, but the characteristics of CSA of MDV may well be similar to those of HVT.

This paper is a preliminary report on a state of dissociation of antigen expression in lymphoid cells from chicks infected with MDV.

A virulent strain of MDV, BC-1 or JM, kindly supplied by Dr. A. Okaniwa (Tanabe Pharm. Co., Ltd.) and Dr. H. Sazawa (Nat. Vet. Assay Lab.) respectively, was inoculated intramuscularly into one-day-old chicks. After appropriate periods, viral antigen (VA) from various organs of the chicks was examined by the direct fluorescent antibody technique. Sera for this technique were obtained from chickens infected with MD and the standard ammonium sulfate method was used for separation of globulins from the sera. Material was labeled with fluorescein isothiocyanate, and unreacted dye was removed from the crude conjugates by gel filtration on Sephadex G-25. Conjugates were subjected to chromatography on DEAE-cellulose and eluted with 0.005 M phosphate buffer, pH 7.0, in 0.1 M NaCl. Normal tissues and lymphoid cells were not stained with these labeled sera. As shown in Table 1, some cells with VA were found in the bursa of Fabricius (BF), spleen and thymus. In some chicks, a very few cells with VA were also found in the liver at an early period. We focused attention on CSA and VA of MDV in

1 A part of this work was presented at the 74th Annual Meeting of the Japanese Society of Veterinary Science at Obihiro, on August 31, 1972.

TABLE 1. *Viral antigen in various organs of chicks inoculated with virulent MDV, detected by the fluorescent antibody technique*

	Days													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Spleen		0/5		2/5		3/3		3/3	1/1				1/2	
Bursa		0/5		3/5		3/3		1/3	1/1				0/2	
Thymus		0/5		1/5		1/3		0/3	1/1				0/2	
Liver		2/5		1/5		0/3		0/3	0/1				0/2	
Bone marrow		0/5		0/5		0/3		0/3	0/1				0/2	
Other organs		0/5		0/5		0/3		0/2	0/1				0/2	

Number of chicks with FA positive cells/Number of chicks examined.

TABLE 2. *Cell surface antigen and viral antigen of MDV in lymphoid cells of spleens from chickens inoculated with virulent strains of MDV*

A) Antigens were examined before cultivation.

	Age (days)				(weeks)				
	4	8	12	16	3	4	7	10	12
VA	++	+	+	+	−	−	−	−	−
CSA	±	±	±	±	+	+	+	+	+
A/B1	5/5	5/5	5/5	5/5	5/5	5/5	4/4	5/5	5/5
A/B2	5/5	5/5	5/5	5/5	5/5	5/5	4/4		

B) Antigens were examined after 11–48 hr cultivation.

	Age (days)				(weeks)		
	4	8	12	16	3	4	7
VA	+	+	+	+	+	+	+
CSA	±	±	±	±	+	+	+
A/B1	5/5	5/5	5/5	5/5	5/5	5/5	2/2

A : Number of chickens with FA positive spleen cells.
B : Number of chickens examined. B1 : Chickens inoculated with MDV, strain JM. B2 : Chickens inoculated with MDV, strain BC-1.

lymphoid cells of the spleen of these chicks. As shown in Table 2A, some lymphoid cells in the spleen showed both VA and CSA of MDV. However 3 weeks or more after infection, some lymphoid cells still showed CSA of MDV but no cells with VA of MDV could be seen (Fig. 1).

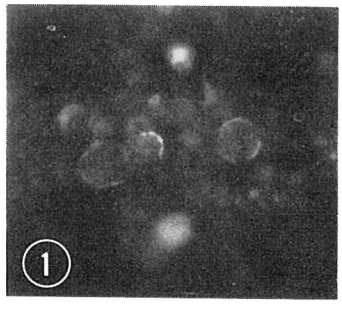

FIGURE 1. *Spleen cells with cell surface antigen of MDV. The cells were prepared from chicks 3 weeks after infection.*

TABLE 3. *Cell surface antigen and viral antigen and agglutinability with concanavalin A of lymphoid cells from various tissues*

No.	Infected with MDV	Age of chicks at Autopsy (W)	Lymphocytic Lesions	Lymphocytes from Lesion			Spleen Cells		
				VA	CSA	Con A[a]	VA	CSA	Con A[a]
1	+	5	Ovary	+	+	+	−	+	ND
2	+	6	Heart	±	±	+	−	+	ND
3	+	6	Liver	±	±	ND	ND	ND	ND
			Ovary	+	+	+			
4	+	7	Liver	+	+	+			
			Spleen	+	+	+	←		
5	+	6	Ovary	±	+	ND	−	+	ND
6	+	6	−				−	+	±
7	−	6	−				−	−	−
8	−	6	−				−	−	−

a Cells agglutinated by 50 μg/ml of concanavalin A, +; by 1,000 μg/ml, ±; not agglutinated by 1,000 μg/ml, −.

The exact proportion of cells with CSA was not determined, but ten or more lymphoid cells with CSA were observed in every field examined under a fluorescent microscope at 200-fold magnification. When these cells were cultured for 11–48 hr, the VA of MDV reappeared and CSA bearing cells increased in number (Table 2B).

The CSA and VA of lymphoid cells constituting a lymphoma of MD were examined. Most of the cells had neither CSA nor VA, but some cells had both VA and CSA (Table 3). However, we could not determine whether all the CSA positive cells had VA. On treatment with concanavalin A at a concentration of 50 μg/ml using a modification of the method of Inbar and Sachs (1969), these lymphoid cells in tumors were always agglutinated clearly, while normal lymphocytes were not (Table 3).

The exact nature of the interaction between lymphocytes and MDV is unknown, but this work demonstrates a state of dissociation of CSA from VA in lymphoid cells in vivo. This suggests that MDV may be intrinsically involved in chicken lymphoid cells.

This work was supported in part by a Scientific Research Grant from the Ministry of Education.

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