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CELL SURFACE ANTIGEN ON QUAIL CELLS INFECTED WITH HERPESVIRUS OF TURKEY OR MAREK'S DISEASE VIRUS

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SUMMARY A virus-induced cell surface antigen(CSA) of Japanese quail embryo fibroblasts infected with either Marek's disease virus(MDV) or cell-free herpesvirus of turkey(HVT) was demonstrated by the fluorescent antibody technique. The CSA of HVT reacted well with anti-MDV serum. However the CSA of MDV only gave a weak positive reaction with anti-HVT serum. In the fluorescent antibody reaction of either the CSA or viral antigen of MDV and HVT, the dilution of homologous blocking serum was well correlated with the blocking index. No cross reaction of the CSA of these two viruses with various other viruses was found. These results indicate that the CSA induced by these two viruses is viral specific. Formation of the CSA of HVT was not inhibited by the presence of cytosine arabinoside, but was completely inhibited by the presence of puromycin. This suggests that production of the CSA of HVT is not dependent on the replication of viral DNA and that the antigen is newly synthesized.

INTRODUCTION

A virus-induced cell surface antigen (CSA) has been demonstrated by several different immunological methods on transformed cells after infection with various oncogenic viruses (Teventhia et al., 1965; Irlin, 1967). CSA was also demonstrated during cytolytic infection with oncogenic viruses as well as nononcogenic viruses, such as poxvirus (Miyamoto and Kato, 1968, 1971; Ueda et al. 1969) or chikungunya virus (Mantani and Igarashi, 1971). Two CSA of cells infected with oncogenic herpes virus have been reported: CSA depending on EB virus was reported by Klein et al. (1966, 1968), and CSA of cells infected with Marek's disease virus (MDV) was demonstrated by Chen and Purchase (1970). The susceptibilities of Japanese quail embryo fibroblasts to MDV and herpesvirus of turkey were demonstrated by Onoda et al. (1970) and Ono et al. (1971), respectively.

The present communication reports the presence and some characteristics of CSA on quail embryo fibroblasts infected with cell-free herpes virus of turkey (HVT, O1 strain) isolated by Ono et al. (1971) and MDV, C2 strain isolated by Kato et al. (1970).

The abbreviations used in this paper are

shown in Table 1.

TABLE 1. *Abbreviations*

CSA:	cell surface antigen
VA:	viral antigen
MDV:	Marek's disease virus
HVT:	herpesvirus of turkey
EBV:	Epstein-Barr virus
CPV:	cowpox virus
VacV:	vaccinia virus
ChV:	chikungunya virus
QUEF or Q:	Japanese quail embryo fibroblasts
DEF or D:	duck embryo fibroblasts
FAT:	fluorescent antibody technique

MATERIALS AND METHODS

1. *Viruses*

The viruses used in this work are shown in Table 2. Three kinds of Marek's disease virus (MDV), C2 strain and two kinds of HVT, O1 strain with different passage histories, were used. Cell-free virus of the HVT O1 strain was obtained from the Kanonji Institute of the Research Foundation for Microbial Diseases, Osaka University in a lyophilized form. Lyophilized virus was resuspended in MEM.

TABLE 2. *Viruses*

1) MDV, HVT

Virus	Strain	Passage history
HVT	O1	D33, Q4[a]
HVT	O1	D61, Q14
MDV	C2	D17, Q14
MDV	C2	D75, Q12
MDV	C2	D12, Q111
MDV	JM	D6, Q3

2) Other viruses

EBV in P3HR-1 cell line obtained from Dr. Y. Hinuma (Kumamoto Univ.)

CPV LBred, VacV Ikeda } obtained from Dr. H. Miyamoto in this Department.

ChV obtained from Dr. M. Mantani in this Department.

[a] Passaged 33 times in duck embryo fibroblasts and 4 times in quail embryo fibroblasts.

2. *Cells and media*

Japanese quail embyo fibroblasts (QUEF) were prepared and cultured as described previously (Onoda et al. 1970). QUEF were used as host cells of MDV and HVT. BHK 21 cells and FL cells were used as hosts of ChV and poxviruses respectively. These cells were cultured in medium consisting of 90% Eagle's minimum essential medium (MEM), 10% inactivated calf serum, 100 units of penicillin and 100 μg/ml of kanamycine. P3HR-1 strain cells derived from Burkitt's lymphoma were kindly supplied by Dr. Y. Hinuma (Kumamoto University). The cells were cultured in a similar medium consisting 80% MEM, 20% calf serum and the same amounts of antibiotics as for FL cells. These cells were grown at 37 C for 4 days and for virus production the cells were transferred to 33 C. Cultures were further incubated at 33 C for 7 days and usually yielded 20 to 25% cells which were immunofluorescent positive for EBV capsid antigen.

3. *Antisera*

As shown in Table 3, 9 sera were conjugated with fluorescein-isothiocyanate (FITC) for the direct fluo-

TABLE 3. *Antisera*

Sera conjugated with FITC for the direct FAT

1. Anti-HVT O1 turkey (No. 1) serum
2. Anti-HVT O1 turkey (No. 2) serum
3. Anti-HVT O1 chicken serum
4. Anti-MDV C2 chicken serum
5. Anti-MDV JM chicken serum
6. Anti-EBV human serum
7. Anti-CPV rabbit serum
8. Anti-VacV rabbit serum
9. Anti-ChV rabbit serum

Nonconjugated sera for the indirect FAT

1. Anti-HVT O1 turkey (No. 1) serum
2. Anti-HVT O1 turkey (No. 2) serum
3. Anti-HVT O1 chicken serum
4. Anti-MDV C2 chicken serum
5. Anti-MDV JM chicken serum
6. Anti-EBV human serum

Secondary sera conjugated with FITC for the indirect FAT

1. Anti-turkey γ-globulin rabbit serum
2. Anti-chicken γ-globulin rabbit serum

rescent antibody technique (FAT). Aliquots of six sera (No. 1–6) out of the nine sera remained unconjugated and were used as unconjugated primary sera for the indirect FAT and two sera were used as secondary sera and conjugated with FITC in the indirect FAT.

1) Anti-HVT sera

a) Two anti-HVT turkey sera were obtained from a normal turkey inoculated with HVT O1 (D7) intraperitoneally. Blood was obtained 60 days after inoculation. Sera having a titer of ×640 against homologous HVT antigen by the indirect FAT were used.

b) Anti-HVT chicken serum was obtained from a RIF free white Leghorn chicken inoculated with HVT O1 (D23). The chicken was raised in a vinyl isolator to prevent infection with MDV. The antibody titer was ×640 against HVT and ×40 against MDV by the indirect FAT.

2) Anti-MDV sera

a) Anti-MDV C2 chicken serum was obtained from a RIF free white Leghorn chicken which had been inoculated with MDV C2 (D12, Q60) and raised in a vinyl isolator. The antibody titer was ×640 against MDV and ×40 against HVT.

b) Anti-MDV JM chicken serum was obtained from a RIF-free white Leghorn chicken which had been inoculated with MDV JM and raised in a vinyl isolator. The antibody titer was ×640 by the indirect FAT.

3) Other sera

Anti-EBV serum which had been obtained from a patient with nasopharyngeal carcinoma, was kindly supplied by Dr. S. Sakai (Osaka Kaisei Hospital). The antibody titer was ×2,560 by the indirect FAT. Anti-CPV rabbit serum and anti-VacV rabbit serum were the same sera used by Miyamoto and Kato (1971). Anti-ChV rabbit serum was the same serum used by Mantani and Igarashi (1971). Anti-turkey γ-globulin rabbit serum and anti-chicken γ-globulin rabbit serum were prepared as described previously (Naito et al., 1970).

4. *Fluorescent antibody technique*

Fluorescein-isothiocyanate conjugated antibodies were prepared as described by Naito et al. (1969) for the direct FAT and by Naito et al. (1970) for the indirect FAT. For the direct FAT, FA conjugates were diluted 4-fold. The staining titer of either anti-HVT FA or anti-MDV FA conjugates diluted was ×8. The FA staining procedure for viral antigen was described previously (Naito et al., 1969, 1970). The staining procedure to demonstrate cell surface antigen was as described by Miyamoto and Kato (1971). To stain monolayers of infected cells, cells were washed three times with Hanks' solution, and immediately covered with FA conjugate. After incubation for 1 hr at 37 C, the samples were washed three times with Hanks' solution, and covered with a drop of mounting medium (90% glycerol and 10% carbonate-bicarbonate buffer pH 9.5). Fluorescence was observed under a Tiyoda fluorescent microscope with a dark field condenser. To stain suspended infected cells, cells were removed from the bottom of the culture glass by treatment with EDTA-trypsin solution (0.08% EDTA and 0.05% trypsin in PBS) for 30 sec, and suspended in Hanks' solution. They were washed three times with centrifugation and incubated for 1 hr at 37 C with FA conjugate. Then they were washed three times with Hanks' solution, and 1 drop of 90% glycerol solution mentioned above was added to the packed cells. To demonstrate CSA using the indirect FAT, the cells were washed three times with Hanks' solution and exposed to unconjugated primary serum for 30 min at 37 C. Then they were rewashed three times with Hanks' solution. The following procedure was as described above.

5. *Chemicals*

Cytosine arabinoside and puromycin were purchased from Nutritional Biochem. Corp. U.S.A. Cytosine arabinoside was used at a concentration of 100 μg/ml, which inhibited both cellular and viral DNA synthesis. Puromycin was used at a concentration of 2 μg/ml.

RESULTS

1. *Appearance of cell surface antigen on cells infected with either MDV or HVT*

QUEF cultures infected with either MDV or HVT were treated with homologous antiviral serum conjugates. Semiannular or annular specific fluorescence along the margin of infected cells was clearly demonstrated, but no fluorescence was observed on uninfected cells. Generally cells with semiannular fluorescence were predominant in the early stage and number of cells with annular and

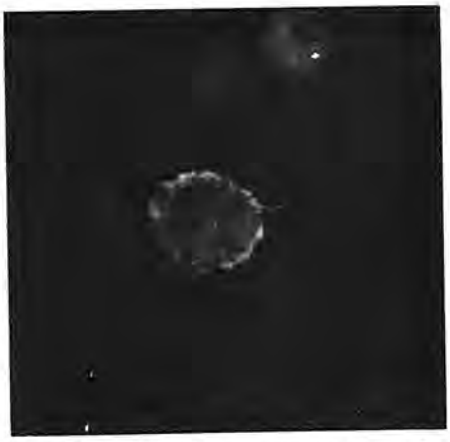

FIGURE 1. *Annular fluorescence on the surface of quail embryo fibroblasts infected with MDV C2. Stained with FITC labeled anti-MDV JM chicken γ-globulin without fixation.*

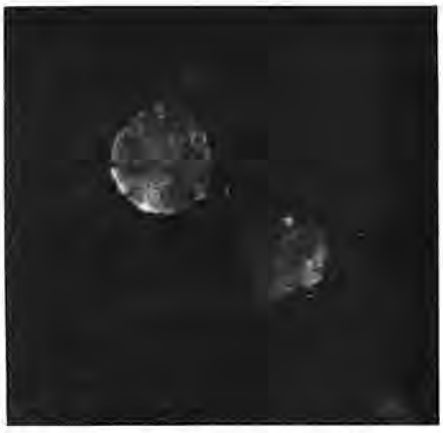

FIGURE 2. *Annular fluorescence on the surface of quail embryo fibroblasts infected with HVT O1. Stained with FITC labeled anti-HVT O1 turkey γ-globulin without fixation.*

granular fluorescence increased later. Groups of cells showing CSA are almost identical with foci of infected cells. Figures 1 and 2 show annular CSA fluorescence on infected cells which were suspended and treated with FA-conjugate.

2. *Cross reactivity of CSA demonstrated with anti-MDV sera and anti-HVT sera.*

The cross reactivities of the CSA and viral antigen (VA) in cells infected with various kinds of MDV, with two kinds of anti-MDV sera were examined. As shown in Table 4, the cross reactivity of the CSA and VA was confirmed. The cross reactivities of three kinds of anti-HVT sera with the CSA and VA of cells infected with two kinds of HVT with different passage histories, were also examined. These antigens reacted similarly with the antisera (Table 5). Then the cross reactivities of the CSA of various kinds of viruses, with antisera against these viruses were examined. As shown in Table 6, no CSA of cells infected with either MDV or HVT was demonstrated with antisera against EBV, CPV, VacV or ChV. The antibody activities of these antisera were high enough to demonstrate homologous CSA. The CSA

TABLE 4. *Cross reactivities of CSA and VA of various kinds of MDV with two kinds of anti-MDV sera*

Antigens	Antisera			
	MDV C2 Chicken		MDV JM Chicken	
	VA	CSA	VA	CSA
MDV C2, D17Q14	+	+	+	+
MDV C2, D75Q12	+	+	+	+
MDV C2, D12Q11	+	+	+	+
MDV JM, D6Q3	+	+	+	+

TABLE 5. *Cross reactivities of CSA and VA of HVT with three kinds of anti-HVT sera*

Antigens	Antisera					
	HVT Turkey No. 1		HVT Turkey No. 2		HVT Chicken	
	VA	CSA	VA	CSA	VA	CSA
HVT O1 D33Q4	+	+	+	+	+	+
HVT O1 D61Q7	+	+	+	+	+	+

of HVT reacted with anti-MDV serum as strongly as the CSA of homologous viruses. However the CSA of MDV only gave a weak positive reaction with anti-HVT serum.

TABLE 6. *Cross reaction of CS antigens of various kinds of virus with various sera demonstrated by the direct FAT*

Antigens	Antisera					
	MDV	HVT	EBV	CPV	VacV	ChV
MDV-QUEF	3+	1+	—	—	—	—
HVT-QUEF	3+	3+	—	—	—	—
EBV in P3HR-1	—	—	3+	—	—	—
CPV-FL	—	—	—	3+	3+	—
VacV-FL	—	—	—	3+	3+	—
ChV-FL	—	—	—	—	—	3+
QUEF	—	—	—	—	—	—

3. *Effect of dilution of the blocking serum on the FA reaction of the CSA and VA of MDV and HVT*

An experiment was carried out to confirm the viral specificity of the CSA of these viruses, as described for the CSA of EBV by Gunven and Klein (1971). Four fold serial dilutions of a blocking serum (anti-MDV JM chicken serum), were allowed to block anti-MDV JM chicken serum conjugate to stain QUEF infected with MDV C2. The samples contained about 40% of infected cells. The range of dilution of the blocking serum was 1:2.5~1:640. The blocking index(BI) was calculated by subtracting the percentage of FA positive cells in the samples exposed to serum from the percentage in the sample exposed to the conjugate alone and dividing the difference by the latter value, as follows.

$$\text{Blocking index (BI)} = 1 - \frac{B}{A}$$

A: Percentage of FA positive, untreated cells.

B: Percentage of FA positive cells exposed to blocking serum.

Percentage was calculated by counting 1,000 cells. The mean value of the BI in two tests is recorded in Fig. 3.

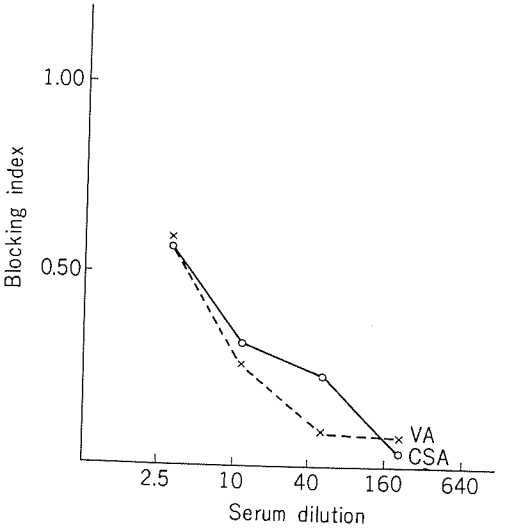

FIGURE 3. *Effect of dilution of the blocking serum on the FA reaction of CSA of MDV. Antigen, QUEF infected with MDV C2; Blocking serum, anti-MD JM chicken serum; Conjugated serum, anti-MDV JM chicken serum.*

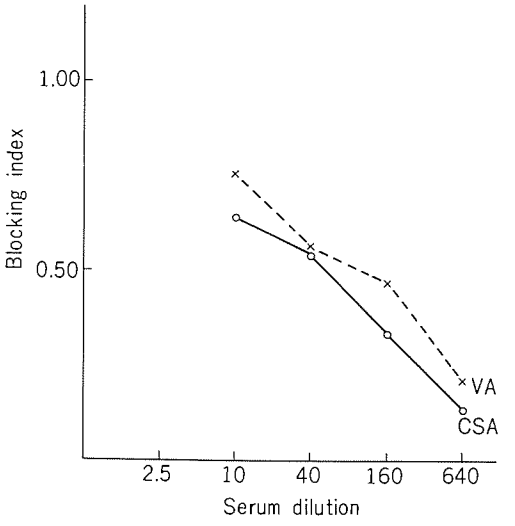

FIGURE 4. *Effect of dilution of the blocking serum on the FA reaction of CSA of HVT. Antigen, QUEF infected with HVT O1; Blocking serum, anti-HVT O1 turkey serum; Conjugated serum, anti-HVT O1 turkey serum.*

A similar experiment was carried out to see the effect of different dilutions of blocking serum on the FA reactions of CSA of HVT. QUEF infected with HVT O1, anti-HVT turkey serum, anti-HVT turkey serum coupled with FITC were used as antigen, blocking serum and conjugated serum, respectively. The mean BI of two tests is recorded in Fig. 4. As shown in these figures, in the FA reaction of either CSA or viral antigen the dilution of homologous blocking serum was well correlated with the BI.

4. *Effects of antimetabolites on the formation of CSA of HVT.*

It is rather difficult to observe the effects of antimetabolites on the formation of CSA induced by MDV, because of the cell-associated nature of the infectivity of MDV. Cell-free HVT having 2.5×10^6 PFU/ml titrated on CEF was used for this purpose. As shown in Fig. 5, QUEF cultures were transferred to medium containing cytosine arabinoside (100 γ/ml) or puromycin (2 γ/ml) and cultured for 3 hr. QUEF cultures (4×10^5 cells/dish) were infected with the HVT O1 strain (5×10^5 PFU/0.2 ml/dish), and after adsorption for 2 hr, they were washed three times with Hanks' solution, and reincubated in medium containing cytosine arabinoside or puromycin. Infected QUEF cultures were also subjected to the same treatments with medium containing no antimetabolites. At intervals, two samples were subjected to the FAT for measurement of the CSA and VA. The average percentages of fluorescent positive cells in the two samples are plotted in Fig. 6 for the experiment with cytosine arabinoside and in Fig. 7 for the experiment with puromycin. Percentage was calculated by counting 1,000 cells in each sample.

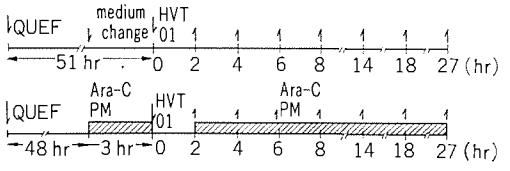

FIGURE 5. *Scheme of experiment on the effects of cytosine arabinoside and puromycin on induction of CSA and of QUEF infected with HVT O1. Ara-c, cytosine arabinoside; PM, puromycin.*

In control infected QUEF cultures in the absence of the antimetabolite, the numbers of cells with CSA and of cells with VA increased gradually during the obsevation period.

In the presence of cytosine arabinoside, the number of cells with CSA in infected QUEF cultures increased gradually in parallel

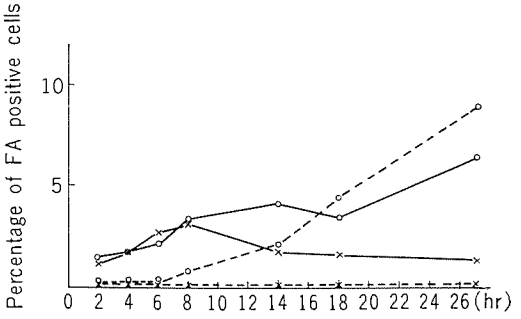

FIGURE 6. *Effect of cytosine arabinoside on CSA and VA of QUEF infected with HVT O1.*
○———○, *CSA (untreated)*
○·····○, *VA (untreated)*
×———×, *CSA (cytosine arabinoside)*
×·····×, *VA (cytosine arabinoside)*

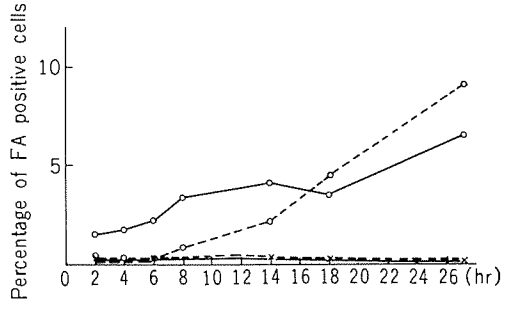

FIGURE 7. *Effect of puromycin on CSA and VA of QUEF infected with HVT O1.*
○———○, *CSA (untreated)*
○·····○, *VA (untreated)*
×———×, *CSA (puromycin)*
×·····×, *VA (puromycin)*

with that in control cells up to 3.1% seen 8 hr after infection and then decreased slowly to 1.2% at 27 hr after infection. Very few cells with viral antigen were observed throughout the experiment (Fig. 6).

In the presence of puromycin, neither cells with CSA nor cells with VA were observed in the infected QUEF cultures during the observation period (Fig. 7).

These results suggest that the CSA of HVT was induced as an early antigen(s).

DISCUSSION

Chen and Purchase (1970) reported briefly on the cell surface antigen (CSA) induced by MDV. In our experiments, the CSA of QUEF infected with MDV and HVT were demonstrated by the FAT using antisera against homologous virus. It seems that CSA is induced on cells infected with virus because the areas of cells showing CSA were nearly identical with the foci of infected cells and the number of CSA positive cells increased in parallel with that of cells with viral antigen (VA). In the FA reaction of either CSA or VA the dilution of homologous blocking serum was well correlated with the blocking index. This also shows the viral specificity of the CSA induced by these two viruses. Partial cross reactivity of VA of MDV and HVT has been demonstrated by the FAT by several investigators (Witter et al., 1970; Sharma et al., 1972; Doi et al., 1971, 1972). In this work we demonstrated the partial cross reactivity of the CSA of MDV and HVT. The cross reactivities of the CSA with other viral antigens of these two viruses are still unknown.

It is difficult to estimate the effects of antimetabolites on induction of the CSA of MDV, because of the cell-associated nature of infectivity. However, this was possible using strain O1 of HVT, because a large amount of cell-free virus can be obtained from infected cells. Induction of CSA on cells infected with HVT was not inhibited by cytosine arabinoside which inhibits viral DNA synthesis, but was inhibited by puromycin, an inhibitor of protein synthesis. It seems certain that the CSA induced by HVT is one of the earliest symptoms of virus infection and is not dependent on virus multiplication. It may be part of the early protein coded by parental DNA of HVT. The effects of these two antimetabolites on the CSA of HVT are similar to their effects on the CSA of other DNA viruses, such as poxviruses (Miyamoto and Kato, 1971).

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