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ANTIGEN COMMON TO A HERPES TYPE VIRUS FROM CHICKENS WITH MAREK'S DISEASE AND EB VIRUS FROM BURKITT'S LYMPHOMA CELLS[1]

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SUMMARY Herpes type virus (Biken C strain) isolated from a chicken with Marek's disease and EB virus from Burkitt's lymphoma cells have at least one antigen in common. This was shown by immunodiffusion tests using the two viruses as antigens and the sera from adult humans whose sera were positive to EB virus by the immunofluorescent technique and from a chicken with Marek's disease as antibodies.

INTRODUCTION

Many strains of herpes type virus associated with Marek's disease (MD) have been isolated (Churchill and Biggs, 1967; Solomon et al., 1968; Nazerian et al., 1968; Churchill, 1968; Yuasa et al., 1968; Rispens et al., 1969; Kato et al., 1970). Chubb and Churchill (1968) and Yuasa et al. (1969) demonstrated a precipitating antigen of a herpes type virus of MD by the agar gel double diffusion technique using sera from birds infected with MD. A previous publication from this laboratory also reported that the antibody seemed to be associated with MD judging by results of the direct fluorescent antibody technique (Naito et al., 1969). Using EB virus from Burkitt's lymphoma cells, Konn et al. (1969) reported that human sera which were positive to EB virus by the indirect immunofluorescence technique yielded specific precipitin lines for EB virus by gel immunodiffusion. Naito et al. (1970) suggested the existence of antigenicity in common to a herpes type virus (Biken C strain) from a chicken with MD and EB virus of Burkitt's lymphoma cells using the indirect immunofluorescence test. Their findings were confirmed in the present work using gel immunodiffusion and it was demonstrated that at least one antigen of herpes type virus of a chicken with MD is identical to an antigen of EB virus from Burkitt's lymphoma cells.

MATERIALS AND METHODS

1. *Antigens*

1) Antigen of herpes type virus from a chicken with MD

Herpes type virus (HTV) (Biken C strain) from

1 Part of this work was presented at the 69th General Meeting of the Japanese Society of Veterinary Science at Azabu Veterinary College, Kanagawa in April 1970.

chicken with MD was isolated in duck embryo fibroblasts (DEF) by Kato et al. (1970) using the method of Solomon et al. (1968). This strain was used throughout.

Precipitating antigen was prepared from DEF infected with HTV (Biken C strain) by the procedure shown in Fig. 1. When the cytopathic effect was estimated to involve at least 50 per cent of the cells in an infected monolayer, the cells were removed by treatment with 0.25% trypsin in phosphate buffered saline. The suspension was centrifuged and the cells were resuspended in phosphate buffered saline (pH 7.2). Then they were subjected to sonication at 10 kc/sec, 150 w for 5 min (Kubota KMS-250). After centrifugation at 3,000 rev/min for 15 min, the supernatant was removed and subjected to ultracentrifugation at 40,000 rev/min for 90 min. The precipitate was resuspended in phosphate buffered saline at 1/40 volume of the supernatant. After sonication at 200 w for 5 min, the suspension was treated with Tween 80-ether by the method of Norrby (1964) as follows. The suspension was treated with a final concentration of 0.1% Tween 80 for 10 min with gentle stirring at room temperature and then a half volume of ether was added and stirring was continued for 15 min in the cold. The phases were separated by centrifugation at 3,000 rev/min for 20 min, and the aqueous phase was withdrawn. Then the ether was removed by evaporation at reduced pressure. Uninfected cultures of DEF were treated in the same way.

2) EB virus from the Burkitt's lymphoma cell line

The P3HR-1 subline of the P3J (Jijoye) Burkitt's cell line was kindly supplied by Dr. Y. Hinuma (Tohoku University). Virus was produced by the method of Konn et al. (1969). Cultures were adjusted to a concentration of 1×10^6 cells/ml. These cultures, which regularly yielded 15% of immunofluorescent positive cells, were incubated at 33 C for 7 days and then washed three times with phosphate buffered saline with centrifugation at 1,500 rev/min for 10 min. These cells were also treated with Tween 80-ether as shown in Fig. 1.

Infected cells
- Sonicated at 150 w, 10 kc/sec, 5 min
- Centrifuged (3,000 rev/min, 15 min)

Sup.
- Centrifuged (40,000 rev/min, 90 min)

Ppt.
- Resuspended in PBS (1/40 volume of the supernatant)
- Sonicated at 200 w, 10 kc/sec, 5 min
- 1/10 volume of Tween 80 (20 mg/ml) added to virus suspension
- 1/2 volume of ether added
- Centrifuged (3,000 rev/min, 20 min)

Aqueous phase : Antigen

FIGURE 1. *Preparation of Antigens*

2. *Antiserum*

The antiserum against chick HTV, used to identify antigens in the gel diffusion test was obtained from a chicken with typical histopathological symptoms of Marek's disease. The serum of this bird was shown to have a high titer of anti-HTV of chicken (Biken C strain) antibody by the indirect immunofluorescent antibody technique (Naito et al., 1970). Human sera were obtained from two healthy subjects (A and B). These sera were shown to have a high titer of anti-EB virus antibody by the indirect immunofluorescent antibody technique (Henle and Henle, 1966 ; Hinuma et al., 1967). Serum from a subject shown to have agammaglobulinemia by electrophoresis was used as control human serum. This serum contained neither γA nor γM, but a trace of γG (1.25 mg/dl). Eleven sera, shown to be free from any anti-HTV of chicken antibody (Naito et al., 1970) were obtained from chickens (150 day old) raised in vinyl isolators. These sera were also used as controls. Normal DEF was used for absorption.

3. *Precipitin test*

The agarose gel used for the double diffusion precipitation test was prepared using the following formula of Churchill et al. (1969).

sodium chloride	4.0 g
disodium hydrogen phosphate	3.45 g
potassium dihydrogen phosphate	0.355 g
agarose	1.0 g
sodium azide	1.0 g
deionized water	80.0 ml

Gel was poured onto glass plates to a depth of approximately 2 mm. Wells of 2 mm diameter were cut out at a center-to-center distance of 6 mm. After addition of antigens and antisera the plates were stood for 48 hr at 37 C in a humidified sealed box and then examined.

RESULTS

Human sera which were positive to EB virus by the indirect immunofluorescence technique were tested against EB virus antigen. A single line was usually observed. Human serum A was tested on the same plate with MD chicken serum and with agammaglobulinemic human serum, against EB virus antigen. An antigen line was formed with MD chicken serum (Fig. 2), although the line between EB virus antigen and the agammaglobulinemic serum was slightly bent. Similar results were obtained with serum B. Similarly, when the three sera were tested against chick HTV as antigen, a line of identity was observed (Fig. 3). In this case, no line or bending of the line was observed with agammaglobulinemic serum.

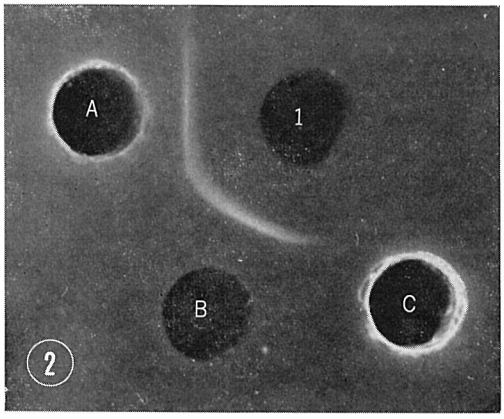

FIGURE 2. *Reaction of EB virus antigen with three sera (human serum A, serum of human with agammaglobulinemia and serum of chicken with Marek's disease).*

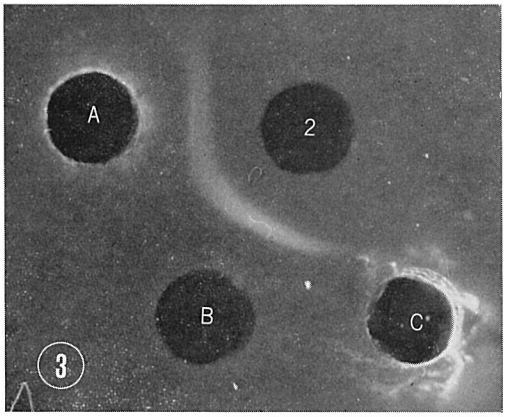

FIGURE 3. *Reaction of herpes type virus of chicken (Biken C strain) with the three sera of Fig. 2.*

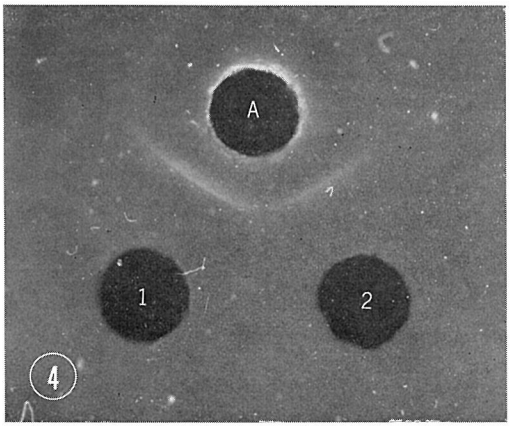

FIGURE 4. *Reaction of human serum A, with EB virus and herpes type virus of chicken (Biken C strain).*

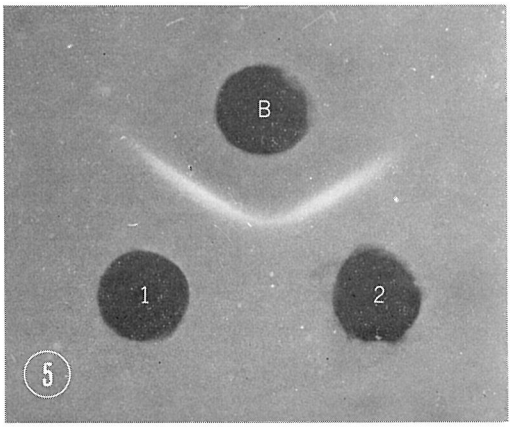

FIGURE 5. *Reaction of serum of chicken with Marek's disease with the two viral antigens of Fig. 4.*

A: Human Serum A
B: MD chicken serum
C: Agammaglobulinemic human serum
1: EB virus antigen
2: Chick HTV antigen

No antigen line was formed by either chicken HTV or EB virus with any of the 11 sera from chickens which had been raised in vinyl isolators. Moreover, no line was formed by these sera against duck embryo fibroblasts similarly treated with Tween 80-ether, as antigen. Fusion of the lines of EB virus and chick HTV as antigens against MD serum and human serum A as antibodies on a single plate was also confirmed (Figs. 4 and 5).

DISCUSSION

Antigenicity common to two herpes type viruses isolated from a Burkitt's lymphoma cell line and from the Lucké adenocarcinoma of a frog has been demonstrated (Fink et al., 1968). Our results deomonstrate unequivocally that at least one antigen present in herpes type virus from a chicken with Marek's disease is identical with an antigen of herpes type virus (EB virus) from a Burkitt's lymphoma cell line. The slight bending of the line between EB virus antigen and agammaglobulinemic serum, shown in Fig. 2, might be due to a trace of gammaglobulin in the serum.

These data suggest that these 3 viruses, which are probably all B group herpes viruses, may have some antigenic relationship.

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