PROPERTIES OF HUMAN SERUM FACTORS PRECIPITATING BOVINE ENTEROVIRUS

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SUMMARY: We investigated the nature of a normal human serum factor(s) precipitating bovine enterovirus, and obtained the following results. (1) The incidence of the precipitating factor in human serum increased greatly at the ages between 2-5. (2) The precipitating factors in most of the sera examined were identified as IgG globulin. (3) A bovine enterovirus was found to possess two distinct antigens resembling N (native) and H (heat stable) antigens of human enteroviruses; normal human serum reacted exclusively with the latter. (4) The majority of human sera precipitating heat stable antigen of bovine enterovirus also reacted with the same antigens of human enteroviruses, coxsackievirus B1, coxsackievirus B5 and echovirus type 1. (5) Absorption experiments of human serum with heated viruses indicated the presence of a common antigen(s) between the heat stable antigen of bovine enterovirus and those of coxsackie- and echoviruses, while no serological relationship was found between the heat stable antigen of bovine enterovirus and those of polioviruses.

From these results it was presumed that the human serum factor precipitating bovine enterovirus is possibly an antibody produced by infection with coxsackie or echoviruses sharing a common antigen(s) with bovine enterovirus.

INTRODUCTION

Many authors have reported that normal human serum contains a substance(s) neutralizing bovine enteroviruses (Moscovici et al., 1961; Klein et al., 1964; Yamada, 1965 b). Recently, we found a factor(s) in human serum precipitating the same viruses.

Bovine enteroviruses, however, are generally considered to be infectious only among cattle; isolation of the viruses from man has never been reported. This study was undertaken to characterize the precipitating factor in sera of humans insusceptible to infection with the virus.

MATERIALS AND METHODS

Serum samples: Sera were collected from healthy persons of various ages residing in Sapporo City and stored at −20 C until use.

Virus strains: K88 strain of type 1 bovine enterovirus isolated in Japan (Yamada, 1965). This study was presented at the 19th Meeting of the Society of Japanese Virologists, 1971.
1965a) was obtained from Dr. S. Yamada of The Chemo-Seroetherapeutic Research Institute, Kumamoto, Japan. The virus was propagated in this laboratory in the culture of freshly trypsinized calf kidney (CK) cells in Eagle’s basal medium without serum.

Human enteroviruses used were coxsackievirus B1 (Cox. B1) (Connecticut 5), coxsackievirus B5 (Cox. B5) (Faulkner), echovirus type 1 (E1) (Farouk), echovirus type 4 (E4) (Pesacek) and polioviruses, type 1 (Mahoney), type 2 (MEF-1) and type 3 (Saukett). These viruses were obtained from NIH, Japan, and grown in monkey kidney stable (MS) cell cultures in the same medium.

**Plaque assay:** Plaque assays of bovine and human enteroviruses were performed on monolayers of CK and MS cells, respectively, according to the method described previously (Urasawa et al., 1972).

**Gel diffusion test:** The procedure of this test was described in detail elsewhere (Urasawa et al., 1968). Bovine and human enteroviruses grown in cell cultures were concentrated by differential centrifugation and used as precipitating antigens of native viruses (infectivity of $10^{10}$ PFU or more/ml). Heated virus antigens were prepared by treating the native viruses at 56°C for 30 min. Rabbit hyperimmune antisera against bovine and human enteroviruses were prepared by repeated intravenous injections with the concentrated native viruses. Virus antigens were allowed to react in agar gel against normal human and immune rabbit sera at 28°C for 48 hr.

**Immunoelectrophoresis:** The method was described previously (Urasawa et al., 1968). Human sera were electrophoresed in agar and then allowed to react with either the precipitating virus antigen or the antiserum against human serum placed in the troughs made in agar gel. The antiserum against whole human serum was prepared in the rabbit by repeated intramuscular injections with human serum with Freund’s complete adjuvant.

**Absorption of human serum with heated virus antigens:** The heated virus antigens of human or bovine enteroviruses were mixed with equal volumes of human serum. After incubated at 4°C for 3 days, the mixtures were centrifuged at 3,000 rpm for 10 min. The supernatant fluid was examined for precipitating activity by the gel diffusion test.

**RESULTS**

**Development of Human Serum Factor Precipitating Bovine Enterovirus at Different Ages**

A total of 111 sera were obtained from persons, aged from 4 months to 70 years, not in contact with cattle in their daily lives. The sera were examined for the activity by gel diffusion tests with bovine enterovirus K88 strain (native virus) as the precipitating antigen. The results are shown in Table I. Three of eleven sera from persons under 1 year of age already possessed the precipitating activity. The incidence in human sera of the precipitating factor increased to 75% at 2-5 years of age and reached the highest level (95%) at the ages between 11-20; thenceforth, the factor was detected at high frequencies up to 70 years of age.
TABLE I
The incidence of K88 virus-precipitating factor in sera from humans of different ages

<table>
<thead>
<tr>
<th>Age</th>
<th>Number of sera</th>
<th>Number of positive precipitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>-1</td>
<td>11</td>
<td>3 (27.3%)</td>
</tr>
<tr>
<td>2-5</td>
<td>20</td>
<td>15 (75.0%)</td>
</tr>
<tr>
<td>11-20</td>
<td>20</td>
<td>19 (95.0%)</td>
</tr>
<tr>
<td>21-40</td>
<td>20</td>
<td>19 (95.0%)</td>
</tr>
<tr>
<td>41-60</td>
<td>20</td>
<td>14 (70.0%)</td>
</tr>
<tr>
<td>61+</td>
<td>20</td>
<td>16 (80.0%)</td>
</tr>
</tbody>
</table>

Precipitation in Agar Gel of Human Serum with Bovine Enterovirus

Selected sera having the precipitating activity and rabbit immune sera against K88 virus (R2, R3) were allowed to diffuse in agar gel against native and heated preparations of K88 virus (Fig. 1). As seen in the upper picture, two precipitin lines appeared between the native virus and the rabbit immune sera, while normal human sera developed a single line with the same virus antigen. The lines formed with the individual human sera fused with each other and with the inner line of the two formed with the immune sera.

In contrast, when the heated virus (10^5.6 PFU/ml) was tested with the immune

![Fig. 1. Precipitation in agar gel of rabbit immune and normal human sera with native and heated K88 viruses.](image-url)
sera, a single precipitin line appeared as seen in the lower picture. The line coalesced with the common line formed with the individual human sera. Experiments with the human and rabbit sera absorbed with normal calf kidney cells gave the same results.

These results may be summarized as follows: (1) A native preparation of bovine enterovirus possesses two distinct antigens as indicated by the two precipitin lines developed between the virus and the immune serum. (2) The antigen corresponding to the precipitin line closer to the antiserum cup is characteristic of native virus particle, while that corresponding to the other line closer to the antigen troughs is characteristic of heat-inactivated virus. These two antigens are tentatively referred to as N (native) and H (heat stable) antigens, respectively. (3) Normal human sera do react only with H antigen of bovine enterovirus in agar gel.

_Immunoelectrophoresis of Human Sera Containing Bovine Enterovirus-precipitating Factors_

The electrophoretic mobilities of the precipitating factors were determined by immunoelectrophoresis using native K88 virus. Figure 2 shows the results with four

![Image of agar gel precipitation patterns](image_url)

Fig. 2. Precipitation in agar gel of electrophoresed human serum with native K88 virus. The patterns were developed by placing rabbit anti-human serum (top) or concentrated K88 virus antigen (others) in the troughs.
human sera and a rabbit immune serum against K88 virus (R3). The pattern developed with the immune serum showed that both antibodies directed against N and H antigens of bovine enterovirus were IgG globulin. All of 30 human sera examined were found to contain the precipitating factors belonging to IgG globulin like those seen in the figure. Two human sera, however, contained the precipitating factors corresponding to β globulins, in addition to that of IgG globulin, as represented by No. 551.

Reactivity of Human Serum to Bovine and Human Enteroviruses

During screening a number of human sera by gel diffusion tests, we noticed that sera capable of precipitating heated K88 virus tended to precipitate heated coxsackie- and echoviruses. To ascertain this point, experiments were made with the heated antigens of bovine and human enteroviruses. A majority of human sera precipitating heated K88 virus (77/84) also reacted with heated Cox. B1 virus, while most sera without the activity against the former (21/26) were also inactive against the latter (Table II A). The same human sera were then examined for reactivity against heated Cox. B5 and E1 viruses (Table IIB and IIC). The results also suggested strongly the presence of a common antigen between the bovine and the human enteroviruses.

TABLE II

<table>
<thead>
<tr>
<th>Common antigenicity between heated bovine and human enteroviruses as demonstrated by gel diffusion test</th>
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<tbody>
<tr>
<td>A</td>
</tr>
<tr>
<td>--------------------</td>
</tr>
<tr>
<td>K88 (H)</td>
</tr>
<tr>
<td>+ -</td>
</tr>
<tr>
<td>Cox. B1 (H)</td>
</tr>
<tr>
<td>(H)</td>
</tr>
<tr>
<td>Cox. B5 (H)</td>
</tr>
<tr>
<td>(H)</td>
</tr>
<tr>
<td>E1 (H)</td>
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<td></td>
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Numerals show the number of serum specimens. K88 (H), etc.: heated virus of K88 strain. +, -: precipitation positive, negative.

Absorption of Bovine Enterovirus-precipitating Factor by Heated Antigens

The antigenic similarity between bovine and human enteroviruses was further studied with a human serum, No. 484 (Fig. 3). Heated K88 virus developed a single precipitin line as shown in Fig. 3A. Heated antigens of four different human enteroviruses (Cox. B5, E1, Cox. B1, E4) also developed a single precipitin line with the serum. Although the lines formed with heated human enteroviruses fused with each other due to the presence of common antigen(s) shared by these viruses (Schmidt and Lennette, 1962; Schmidt et al., 1965; Conant et al., 1966; Forsgren, 1968), they spurred over the line formed with heated K88 virus. This result was further analyzed in absorption experiments (Fig. 3B-E). Absorption of human serum with heated K88 virus (HS+K88 (H)) removed the reactivity against the same virus,
leaving the activity to precipitate heated antigens of human enteroviruses. On the contrary, human sera absorbed with heated human enteroviruses (HS+B5(H), etc.) were unable to precipitate not only these viruses but also heated K88 virus. The results indicate that heated K88 virus shares an antigen(s) with heated coxsackie- and echoviruses, and that human serum contains antibody against the antigen(s) specific to heated human enteroviruses in addition to antibody against an antigen(s) held in common by the heated bovine and human enteroviruses.

The antigenic relationship between heated K88 and polioviruses was investigated. As seen in Fig. 4A, the lines produced by heated polioviruses (type 2 and 3) intersected or spurred over the line produced by heated K88 virus. Absorption experiments also seemed to confirm the lack of the antigenicity common between the heated antigens of K88 and of polioviruses (Fig. 4B-D).
DISCUSSION

The serum factor reacting with bovine enterovirus in the gel diffusion test appeared in persons aged between 2-5 years and most of the precipitating factors were IgG globulin. In addition, the precipitating factors were found to react only with the heat stable antigen of the virus. These results suggest that the factors may have been formed in response to infection with some agents serologically related to the virus antigen. As human enteroviruses were suspected strongly of being such agents, the antigenic relationship between heated bovine and human enteroviruses was examined in the present study.

Preparations of human enteroviruses, in general, are known to contain two distinct antigens as revealed by complement fixation and gel diffusion tests: N (or D) antigen associated with the infectious complete particle and H (or C) antigen characteristic of the noninfectious empty capsid (Roizman et al., 1958; LeBouvier, 1959; Schmidt et al., 1963; Forsgren, 1969). Similarly, a preparation of bovine enterovirus grown in tissue culture was also shown to contain two distinct virus particles, an infectious virion and an empty particle (Johnston and Martin, 1971). These two particles, from the results with human enteroviruses, seem to correspond to the antigens tentatively designated as N and H in Fig. 2.

While it has been reported that H antigens of human enteroviruses are identical
or closely related to one another (Schmidt and Lennette, 1962; Schmidt et al., 1965; Conant et al., 1966), the present study revealed possible common antigenic structures among H antigen of bovine enterovirus and those of coxsackie- and echoviruses. These results, together with the failure in isolating bovine enteroviruses from stool of children (Urasawa, unpublished data), strongly indicate that the factors precipitating bovine enterovirus are possibly antibodies formed by infections with various human enteroviruses.

ACKNOWLEDGEMENT

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REFERENCES