NOTE

Interference Patterns between Strains of Bovine Viral Diarrhea-Mucosal Disease (BVD-MD) Virus

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ABSTRACT. Strains of bovine viral diarrhea-mucosal disease virus isolated in Japan were examined on the ability of interference. Among the strains, one strain (No. 12) was non-cytopathogenic and the other three strains (Nose, So and Tokachi strains) were cytopathogenic. No. 12 strain strongly interfered with the Nose strain but not remarkably with the So and Tokachi strains.—KEY WORDS: BVD-MD virus, interference.

Bovine viral diarrhea-mucosal disease (BVD-MD) virus has been isolated in tissue cultures in many places in Japan. The first isolated strain, No. 12 strain was non-cytopathogenic (non-CP) and it was detected by chance in bovine testicular (BT) cell culture using END method [6]. The Nose strain was cytopathogenic (CP) and isolated in BT cell culture from the feces of a cattle with a chronic alimentary illness [4]. The So strain was CP and it was isolated from a severe chronic case (This strain was unpublished). The Tokachi strain was CP and isolated in bovine kidney (BK) cell culture from the lung of a cattle affected with mucosal disease [3]. These four strains showed same characters in antigenicity (Itôh et al.: Presentation at the 95th meeting of the Japanese Society of Veterinary Science, Tokyo, 1983), but the character of cytopathic effect (CPE) in BT cell culture was different each other [9]. In the present study, we examined an interfering ability of the non-CP strain against the three CP strains.

The No. 12 and Nose strains were passaged 25 and 10 times in BT cell culture, respectively. The So and Tokachi strains were passaged 5 times in the BK cell culture, and once in BT cell culture, respectively. BT cell culture used in this experiment was the 5- to 10-passaged cell culture in Eagle's minimal essential medium containing 10% bovine serum without antibody to BVD-MD virus, 10% tryptose phosphate broth (Difco), 1% of 7% sodium bicarbonate and antibiotics.

Interfering assay was performed as follows. The infected cell culture fluid containing 1×10^4.5/0.1 ml/median tissue culture infective dose (TCID50) of the virus of non-CP strain (No. 12 strain) was serially diluted by 10-fold steps from 10^{-1} to 10^{-6} in the culture medium. One-tenth ml of each dilution was put into each well of horizontal line in three 96-well microplates per dilution. Then 0.1 ml of BT cell suspension containing 4×10^5 cells was added to each well. The microplate cultures were incubated at 37°C in a humidified CO2-incubator. Four days after incubation, the cultures were challenged with each CP strains (Nose, So and Tokachi strains) respectively as follows. The infected cell culture fluids containing 10^4.5 TCID50/0.1 ml of each CP strains were serially diluted by
Fig. 1. Interference of cytopathic effect to CP strains in BT cell culture infected with the No. 12 strain of BVD-MD virus.

Filled area: Interfering area of cytopathic effect by the No. 12 strain. 10^{4.5}TCID_{50}/0.1 ml/No. 12 strain was 10 fold diluted 10^{-1} to 10^{-6}, and simultaneously inoculated with 4×10^{5}/0.1 ml BT cell (horizontal line). Four days after inoculation the cultures were challenged with 10^{4.5}TCID_{50}/0.1 ml CP strains, these were 4 fold diluted 10^{-1} to 10^{-10} (vertical line).

*: n showed virus dilution index.

Fig. 2. Growth curves of three CP strains in BT cell culture infected with or without the No. 12 strain of non-CP BVD-MD virus.

--- --- : Growth curves of CP strains in BT cell culture without infection with the No. 12 strain.

--- --- : Growth curves of CP strains in BT cell culture infected with the No. 12 strain.
4-fold steps from $4^{-1}$ to $4^{-10}$ in the culture medium. One-tenth ml of each dilution was inoculated to each 6 wells of vertical line of the microplates after the culture medium had been removed. The cultures were incubated at 37°C in the CO₂-incubator, and observed for CPE daily for 7 days.

The effect of the challenge time with CP strains on the interference was examined by comparing growth curves of CP strains in BT cell cultures infected with non-CP strain. BT cell cultures were inoculated with 0.1 ml of culture fluid containing $10^5$ TCID₅₀ of the virus (moi 1) of non-CP strain. At the same time and 2 and 4 days after inoculation with non-CP strain, the BT cell cultures were inoculated with 0.1 ml of culture fluid containing $10^5$ TCID₅₀ of CP strains each. The samples for virus titration of CP strains were collected 1, 3, 5 and 7 days after inoculation with CP strains. The virus titration of CP strains was done by the micro method [10].

The results of the interfering assay are shown in Fig. 1. The No. 12 showed a clearly interference with CPE of the Nose strain. Interference with the CPE of the So and Tokachi strains was seen when challenged with a small amount of the CP viruses, but not with a large amount of the viruses.

The results of the growth tests of CP strains in BT cell culture infected with non-CP strain were presented in Fig. 2. No interference in the virus growth of CP strains was demonstrated when non-CP strain were inoculated simultaneously in BT cell cultures, but when CP strains were inoculated 2 days after inoculation with non-CP strain, the virus growth of the Nose and So strains was inhibited strongly and slightly, respectively. That of the Tokachi strain, however, was not so much inhibited. When CP strains were inoculated at 4 days after inoculation with non-CP strain, the virus growth of the Nose strain was inhibited, but not those of the So and Tokachi strains.

It has been demonstrated that CP and non-CP strains share the same antigenicity [7]. On the contrary, it has been confirmed that the biological character between CP and non-CP strains is quite different in the growth of virus and in the susceptibility to actinomycin D [5]. Moreover, it has been reported that the effects of acriflavine and proflavine sulfate to the growth of CP strains differ among the strains [1, 2, 8]. From these reports and our data, it is suggested that the different biological characters exist in BVD-MD virus in spite of no antigenic difference. The relation of biological character to pathogenicity is the subject to the studied.

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REFERENCES
要約

牛ウイルス性下痢・粘膜炎（BVD・MD）ウイルス株間の干渉能の違い（短報）：白井淳賢・田中義夫・堀内貞治（農林水産省家畜衛生試験場）——日本で分離されたBVD・MDウイルスについて、細胞病原性株（No. 12株）の細胞病原性株（Nose、曾於、十勝株）に対する干渉能を調査した結果、No. 12株によって、Nose株は強く干渉されたが、曾於株および十勝株はそれほど強い干渉を受けなかった。