BRIEF NOTE

Antigenic Relationship between the Tokyo and the Miami Strains of Equine Influenza Subtype 2 Virus

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The first outbreak of equine influenza (EI) infection in Japan was recognized during the period December 1971 to January 1972 [1, 6]. No evidence of the disease had been found before then [2, 6]. The etiological agent of this epizootic was identified by hemagglutination-inhibition (HI) and neutralization tests with chicken or ferret antiserum as the subtype 2 of EI virus [6, 7]. However, the isolate, A/equine/Tokyo/71 (Tokyo) strain, was not completely identical to the prototypic A/equine/Miami/63 (Miami) strain of the subtype 2, since antibody responses of convalescent horses were 2 to 16 times higher against Tokyo strain than against Miami strain used in the HI tests [6, 7]. The same phenomenon was also demonstrated on the EI virus (Heq2 Neq2) strain isolated during the course of epizootics occurred in Brazil in 1969, by means of the HI tests with postinfection horse sera. At that time it was reported that a significant antigenic drift might have occurred within the EI subtype 2 viruses [9]. In the present study, the antigenic relationship between the Tokyo and Miami strains was assessed by cross HI tests with antisera of the immunized rabbits with these strains.

The A/Hong Kong/68 strain of human influenza virus is also included for comparative purposes because of its well-known close relation of antigenicity of the subtype 2 of EI virus [4, 5].

HI test was performed by microtiter method using a 2-fold dilution of serum, 8 hemagglutinating units of virus antigen in 0.025 ml each, and 0.5% chicken erythrocytes in 0.05 ml volume. The serum samples used for the test were treated with M/60 potassium periodate (1.5 volumes: 1 volume of inactivated serum at 56°C for 30 minutes) at 20°C for 1 hour, and excess periodate was neutralized with 6% glycerol saline (0.5 volume) at room temperature for 30 minutes. An additional treatment was performed with commercial Receptor Destroying Enzyme concentrated 3 times (1 volume: 3 volumes of periodate-treated serum) at 37°C overnight. The treated serum was inactivated again at 56°C for 30 minutes and absorbed with chicken erythrocytes to remove natural agglutinins against the cells. The HI antigen was fresh allantoic fluids from 10-day-old embryonating chicken eggs infected with the virus strains used. HI antibody absorption tech-

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nique used in this study was essentially the same as that described by Jensen and Francis [3]. Then the minimal volume of ghost-virus complex which would completely remove the 32 units of homologous HI antibody in a single absorption was defined as 1 absorbing unit (AU).

Cross HI tests on Miami, Tokyo and Hong Kong strains were performed with the antisera obtained at weekly intervals from the immunized rabbits with each of the strains, using two rabbits per strain (Fig. 1). In both of the rabbits immunized with Miami strain the titers of HI antibody against the Miami and Tokyo strains are changed with almost the same level throughout the test period. A slight relation of the Hong Kong strain was shown on or after the second immunization. Antibody responses of the immunized rabbits with Tokyo strain were 2 to 4 times higher against the Tokyo strain than against the Miami strain in both rabbits. A significant cross-reaction was also observed with Hong Kong strain after the second immunization. The rabbits immunized with Hong Kong strain had higher HI titers of 1:128 to 1:1024 against the homologous strain during the experimental period, whereas only one rabbit had cross HI titer of 1:32 against each of the heterologous strains after the second immunization.

Fig. 2 shows the result of cross HI tests with antisera absorbed with each strain of the virus. These antisera were collected as early as 1 week after the first immunization because the earlier IgG antibody to human influenza virus was much more strain specific than the later antibody in rabbits experimentally immunized with the virus [10]. Absorption of the anti-Miami serum with Miami or Tokyo strain almost completely removed heterologous as well as homologous antibodies in the absorption with 1 AU,

Fig. 1. Antibody response of rabbits immunized with Miami, Tokyo or Hong Kong strain

Remarks.

Antibody titer against Miami (●), Tokyo (□) and Hong Kong (▲) strains.

Arrows indicate the times of immunizations with 5,120 to 10,240 hemagglutinating doses of virus for each time.

and completely removed them by the absorption with 2 AU. Absorption of the anti-Tokyo serum with Tokyo or Miami strain removed all antibodies that heterologously react with Miami strain in the absorption with 1 or 2 AU. However, the Tokyo serum still reacted to high titer with homologous strain even after absorption with 2 AU of Miami strain, although absorption of the serum with 2 AU of Tokyo strain totally removed all homologous antibodies. Absorption of the Miami and Tokyo sera with 1 or 2 AU of Hong Kong strain did not lead to the significant reduction of the homologous antibody. The anti-Hong Kong serum showed the significant reduction of HI titer in the absorption with 1 AU of Hong Kong strain, and the negative in titer after the absorption with 2 AU.

Fig. 2. Cross absorption tests of rabbit antisera

Remarks.

*: Outlined areas indicate HI titer against Miami (M), Tokyo (T) and Hong Kong (H) strains with unabsorbed sera and hatched areas represent the titer after absorption with 1 or 2 AU (1 AU=complete absorption of homologous HI titer of 1:32).

No change of HI titer was seen by the absorption of anti-Hong Kong serum with the Miami or Tokyo strain. No cross correlation between the Hong Kong strain and the Miami or Tokyo strain in the HI tests was observed. In these cross HI tests, the antigenicity of Tokyo strain was uniquely shown as compared to the Miami strain. Thus, both antibodies against Miami and Tokyo strains in the antiserum to Miami strain, and all of the antibodies reacting with Miami strain in the Tokyo antiserum, were easily absorbed either with Miami or Tokyo strain. In the antiserum to Tokyo strain, Tokyo antibody was also removed by the absorption with homologous virus, but not with Miami strain. The similar antibody response of rabbits hyperimmunized with hemagglutinin subunits of the A/Port Chalmers/73 strain (H3N2) of human influenza virus was recently reported by Laver et al. [8]. That is, absorption of Port Chalmers antiserum with particles of Hong Kong virus (H3N2) removed all of the antibody reacting with the Hong Kong strain in HI tests, but left behind 62% of the antibody reacting with Port

Chalmers strain. Absorption of this serum with Port Chalmers virus totally removed all detectable antibodies. From the results they concluded that the residual activity was directed against the "specific" determinants on the hemagglutinin subunits of Port Chalmers virus. Then the present result suggests that the Tokyo strain shared the "common" antigen with the Miami strain and the "specific" antigen which differed from the Miami strain. No remarkable difference in the antigenic correlation between the Miami and Tokyo strains was observed in comparison with Hong Kong strain in cross HI tests.

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