NOTE

Hemagglutination-Inhibiting Antibody Sensitive to 2-Mercaptoethanol in Cattle Infected with Ibaraki Virus

Shuichi TOKUHISA, Yuji INABA, Kunihiko SATO, Yasuo MIURA, Noboru KANEKO, and Minoru MATUMOTO

National Institute of Animal Health, Tsukuba, Ibaraki 305, and
1) Kitasato Institute, Minato, Tokyo 108

(Received 14 April 1983/Accepted 7 July 1983)

Ibaraki virus, a member of the Orbivirus genus [3], has been known to cause a bluetongue-like disease of cattle in Japan [1, 4]. Recently we reported the hemagglutination (HA) and HA-inhibition (HI) with Ibaraki virus [6, 7]. In the present study we applied the HI test to study serological responses in cattle infected with Ibaraki virus and further studied the effect of 2-mercaptoethanol (2-ME) on HI titers.

The HI test was carried out by the microtiter method using bovine erythrocytes. The method was described elsewhere [6]. The HA antigen was concentrated by ultrafiltration from culture fluid of HmLu-1 cells infected with Ibaraki No. 2 strain [2, 5]. Sera for the test were inactivated at 56°C for 30 min. For 2-ME treatment 0.2 ml of the serum was mixed with 0.2 ml of a hypertonic diluent and 0.2 ml of 0.2 M 2-ME, and incubated at 4°C for 24 hours. The hypertonic diluent used consisted of 0.6 M NaCl, 0.2 M phosphate buffer at pH 7.5 and 0.2% bovine serum albumin. The incubated material was then treated with 0.5 ml of 25% (w/v) kaolin at room temperature for 30 min, and centrifuged at 1,000 xg for 10 min. The supernatant fluid was used as a 5-fold diluted serum in HI test. The control serum was processed in the same manner after mixing with distilled water instead of the 2-ME solution. Erythrocytes were suspended in 0.2 M phosphate buffer at pH 7.5 containing 0.2% bovine serum albumin. HA antigen and sera were diluted with the hypertonic diluent described above. The serum-antigen mixtures were incubated at 4°C overnight, mixed with erythrocyte suspension, and incubated at 4°C for 4 hours before the results were read. The HI titer was expressed as the reciprocal of the highest serum dilution showing complete HI with 4 hemagglutinin units of antigen.

Neutralization (NT) test was carried out in HmLu-1 cell cultures with the Ibaraki No. 2 strain by the microtiter method [6]. The serum-virus mixtures were incubated at 37°C for 60 min. The virus does was 100 TCID₅₀ per well. The NT antibody titer was expressed as the reciprocal of the highest serum dilution inhibiting cytopathic effect in at least one of the 2 wells.

Cattle inoculated subcutaneously with Ibaraki virus rapidly developed NT and HI antibodies as shown in Fig. 1. No. 1
animal was inoculated first with the vaccine strain derived from the Ibaraki No. 2 strain [2] and 4 weeks later with the Ibaraki No. 2 strain. No. 2 animal was infected only with the Ibaraki No. 2 strain. In these animals HI antibody was first detected on the 11th and the 14th day after infection, respectively, and the titer increased rapidly, reaching a plateau 3 weeks post-infection. When the sera were treated with 2-ME, HI antibody became 2-to 8-fold lower in titer and hence was detected 3 or 4 days later than that of the respective untreated sera. Thereafter both the titers remained constant for about 10 weeks with subsequent gradual decline. The NT titer began to rise in the second week after infection, reached a plateau 3 to 4 weeks post-infection, and thereafter remained almost constant.

Table 1 summarises effect on HI titers of the 2-ME treatment of sera obtained at intervals from 5 cattle infected intravenously or subcutaneously with the Ibaraki No. 2 strain or the vaccine strain.
The inoculated animals developed fever and leukopenia but no other symptoms. Viremia and rise in NT titer were demonstrated in all the animals. HI titers of untreated sera began to rise 10 days after infection or somewhat later and reached a plateau of 640 to 2560 three to four weeks post-infection. The 2-ME treatment decreased the HI titer 2 to 8-fold. Thereafter both the titers remained constant with the titer after the 2-ME treatment being slightly lower. After 90 days postinfection both the titers declined and no titer difference was shown between the treated and untreated sera. These results indicate that the initial phase of HI antibody response represents the IgM response and the later response a change to IgG.

The HI test seems to be more useful than the NT test in the serological diagnosis of Ibaraki virus infection in cattle, as HI antibody became detectable earlier than NT antibody. The 2-ME treatment of test sera may also be helpful to know the time of infection. The HI test is simple enough for the routine use. Furthermore, the HA antigen is very stable, when lyophilized and can be stored for more than one year without any titer loss [7].

References