ホモロガス抗原を用いたTCND免疫血清のHI価増強について

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BRIEF NOTE

Enhancement of Hemagglutination Inhibition Titers in Tissue-Culture Newcastle Disease (TCND) Vaccinated Chicken

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A live tissue-culture Newcastle disease (TCND) vaccine was developed by Bankowski from the California 11914 strain of Newcastle disease virus (NDV) [1, 2]. One of the advantages of this vaccine is that it gives long-term duration of protection against challenge after only one or two immunization. Some investigators, however, have showed that TCND vaccine is a poor hemagglutination inhibition (HI) antibody producer, though many birds with no or low HI titers were resistant to challenge [3, 4].

In Japan, evaluation of the immune status of chickens vaccinated with NDV vaccine, including TCND, is performed mainly by HI test. The antigen used for the HI test is made from the Ishii strain of NDV [7], and available commercially.

During an investigation on the HI antibody response of chickens to TCND vaccine, it was necessary to enhance the sensitivity of the HI test for detection of the antibody produced by TCND vaccine in chicken serum. A study was therefore made to develop an improved, simple and reproducible method to show the enhanced HI titers.

Two antigens were used for comparision of antigenicity in the HI test. One (Ishii antigen), prepared by treating allantoic fluid infected with Ishii strain virus [7] with ether and potassium periodate, was obtained commercially as a freeze-dried product. The other (TCND antigen) was prepared from allantoic fluid of 10-day-old chicken embryos infected with TCND strain (obtained from Eli Lilly & Co., Indianapolis, USA). The pooled antigen suspensions were tested for their hemagglutinability. If there was a positive titer of $\geq$1:64, the fluid was treated with formalin and glycerin by the procedure described in *Methods for the Examination of Poultry Biologics* [5].

HI test was carried out by the standard microtiter method [6] with 2-fold dilutions of serum and 4 units each of Ishii and TCND antigens simultaneously. The reciprocal of the highest dilution of serum that inhibited hemagglutination completely was regarded as the HI titer of the test serum.

Serum neutralization (SN) test was performed in chicken kidney cell cultures with TCND as the antigen. Equal volumes of virus and 4-fold dilutions of heat inac-

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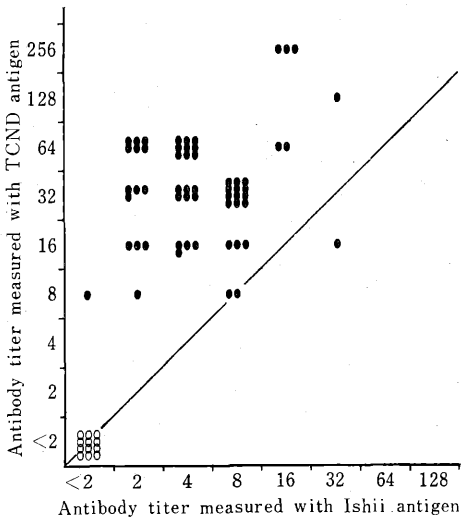
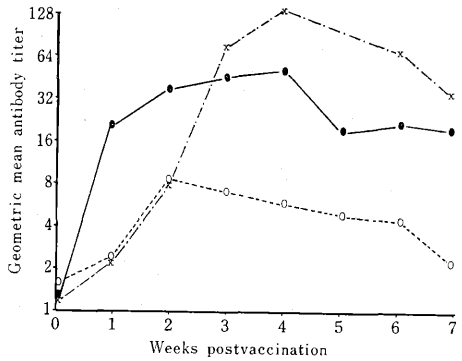
Fig. 1. Comparison of HI titer in TCND vaccine immune serum



Remarks. ●: Vaccinated chicken.
○: Unvaccinated control chicken.

Fig. 2. Immunological responses to TCND vaccine in susceptible birds



Remarks.
●—●: HI titer measured with homologous (TCND) antigen.
○---○: HI titer measured with heterologous (Ishii) antigen.
x—•—x: Serum neutralizing titer.

tivated serum were incubated for 1 hour at 37°C, and then 0.1 m*l* of this mixture was inoculated into each of 4 tubes. The SN titer was defined on the 5th day as that initial dilution of serum inhibiting replication of a 75–175 median tissur-culture infectious dose of this virus. The average of HI and SN titers were expressed by a geometric mean.

Antisera were obtained from ND antibody-free chickens, 3 weeks of age, intramuscularly immunized with TCND virus.

Enhancement of HI titers of the TCND immune sera by use of TCND antigen is showed in Fig. 1. HI titers of the serum with homologous (TCND) antigen was higher than that obtained with commercial heterologous (Ishii) antigen. Fifty-two out of 58 sera gave a 4-fold or more rise in titer with a statistically significant difference (P less than 0.01 by the t-test).

Fig. 2 shows the average antibody titer in a group of 10 chickens immunized by the intramuscular injection of TCND vaccine. Serum samples were collected at weekly intervals for a period of 7 weeks after vaccination and assayed for HI and SN antibodies.

The HI antibody titer measured with TCND antigen reached an average of 1:40 in 3 weeks postvaccination and remained at about that level for the rest of test period. On the contrary, on measurement with Ishii strain of heterologous antigen, a peak was detected in the 2nd week postvaccination, after which the titer decreased. The close relation was observed between SN and HI measured with the TCND antigen, but SN titer was considerably higher than the HI titer since 3 weeks postvaccination.

Observation that TCND vaccine was a poor HI producer might be carried out using strains other than TCND [3, 4]. The data obtained in the present study indicated that the increase of the HI titer against TCND immune sera with TCND antigen is 4-fold or more greater than that obtained with the Ishii antigen conventionally used in Japan.

The reason for the enhancement of HI titer obtained in the homologous reaction with TCND antigen and antibody is not clear. Existence of significant antigenic differences is greatly difficult to explain it. More detailed experiment is neessary in the future according to Webster, who showed that anti-influenza virus rabbits sera gave high titer measured with the only homologous antigen, but not with the other 4 antigens of the same type was due to difference of avidity of the serum [8].

Summury: The sensitivity of the hemagglutination inhibition (HI) test for tissue-culture Newcastle disease (TCND) vaccinated chicken sera increased significantly by use of homologous antigen. The antigen was useful in detecting and estimating immunological response to TCND vaccine in HI test.

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