Transformation of antibody of Japanese encephalitis from IGM to IGG in experimental infected hen and transmission of IGG from hen to chicks. (epidemiological study on Japanese encephalitis. 37)

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Transformation of antibody of Japanese encephalitis from IGM to IGG in experimental infected hen and transmission of IGG from hen to chicks. (epidemiological study on Japanese encephalitis. 37)*

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Abstract

Transformation of Japanese encephalitis antibody from IgM to IgG in the sera of the experimental infected chicks with Japanese encephalitis virus and transmission of IgM or IgG from hen to chicks were examined by the gel filtration on Sephadex G-200 column. The following results were obtained. 1. Titer of hemoagglutination inhibiting antibody rose on seven days after inoculation of mouse brain homogenate infected with Japanese encephalitis, and that increased rapidly after the second inoculation of Japanese encephalitis. The maximum peak of antibody titer attained on 35 days after the first inoculation, on 7 days after the second inoculation and it maintained for a period of 2 months then decreased. Viremia was detected till 6 hours after the first inoculation. 2. IgM antibody by gel filtration appeared on 7 days after the first inoculation, kept on rising, reached the peak on 35 days after the first inoculation, then decreased, and disappeared on 120 days. IgG antibody appeared about 2 weeks after the IgM antibody appearance, and the titer of IgG antibody became higher than that of IgM antibody on 35 days after the first inoculation, then decreased gradually, and showed 1 : 16 of titer of peak on 150 days by gel filtration. 3. We could obtain the chicks by fertilization from experimentally infected hen, having IgM and IgG of hemoagglutination inhibiting antibody of Japanese encephalitis. And the localization of antibodies in the sera of its chicks was determined by Sephadex G-200 gel filtration. And IgG antibody was detected in chick serum, though IgM antibody was not detected by this method.

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TRANSFORMATION OF ANTIBODY OF JAPANESE ENCEPHALITIS FROM IGM TO IGG IN EXPERIMENTAL INFECTED HEN AND TRANSMISSION OF IGG FROM HEN TO CHICKS

(EPIEMIOLOGICAL STUDY ON JAPANESE ENCEPHALITIS 37)

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The potential role of wild birds in the epidemiology of Japanese encephalitis has been studied by experimental infection of Japanese encephalitis. Kitaoka, et al. (1-2) and Hammon, et al. (3) showed independently that virus regularly appeared in the blood, following inoculation of wild and domestic birds with Japanese encephalitis virus in low mouse passage. Buescher, et al. (4-5) described in detail about the time of onset, magnitude and duration of viremia of chicks. They also studied the antibody appearance of black-crowned night herons, little egrets and plumed egrets and concluded that the black-crowned night herons played the most important role among herons. They described that hemoagglutinin inhibiting (HI) and neutralizing (NT) antibodies of hen infected experimentally and those of heron infected naturally transferred to chick as maternal antibody. We (6) recognized that sera of plumed egrets showed high ratio (41%) of HI positive reaction and plumed egrets can also be one of the amplifier of Japanese encephalitis.

We (6) found the presence of naturally infected hens by HI reaction in 1967 and the positive rate was 7 out of 27, and 6 out of 7 positive serum showed 2-ME positive reaction, suggesting that hen was one of the infectious sources of Japanese encephalitis. The HI antibody of hen infected naturally was also transferred to chicks and disappeared in the chick serum after hatching, and half life of the chick HI antibody was four days (7).

In this experiment, we investigated transformation of IgM to IgG in the serum of naturally infected hen, and also studied whether IgM or IgG transferred from hen to chick. This report described briefly our results.
Virus: Japanese encephalitis virus (JaGAr strain) has 45 passage in mouse brain. The seed stock contained $10^{8.7}$ intraperitoneal, LD 50/g of brain tissue.

Hen: Susceptible hens, 60 days after hatching of white leghorn strain, were inoculated twice with $10^{6.7}$ LD 50 subcutaneously at one time. Serum samples prior to infection had no detectable HI antibody at a dilution of 1:2. Serum samples were taken before inoculation, and at 28, 35, 89, 120 and 150 days following initial inoculation.

Tests for circulating virus were performed on samples of blood obtained on the first-, second-, third-, seventh-postinoculation hours.

Chick: Hens, 3 months of age, were bred with non-immune roster, 14 days prior to this experiment, thereafter, hens were inoculated with $10^{6.3}$LD 50 of JE virus subcutaneously. The progenies of hen were hatched from the eggs born 7 to 10 days after inoculation of hen. Bloods of chicks were taken by cardiac puncture one day after hatching. Each pooled blood was collected from 3 chick sera, and 3 pooled sera were prepared from 9 chicks. The sera of hen and chick applied Sephadex G-200 column, and also tested for HI antibody to JE virus in gel filtrate. Immunological reaction: The titration of HI antibody of JE was done by the method described by CLARKE and CASALS (8). The titration of 2-ME sensitive antibodies in HI reaction was done by employing the method of ANN. SCHLEU-derberg (9).

Detection of viremia: Blood was obtained by heparin wetted syringes inoculated intracerebrally (0.02ml) into 6-8 weanling mice. Virus was considered to be present in blood only when at least half the mice exhibited specific sign of infection within 14 days of inoculation.

RESULTS

Viremia and antibody responses:

Animals were infected by two dosage schedule. On 28 days after first inoculation, animals received booster inoculation. In animals inoculated with Japanese encephalitis virus, viremia was recognized to the six postinoculation hours. On 7 days, animals responded the detectable HI antibody. And on 35 days (on 7 days after second inoculation), HI titer reached maximum (1:2560) and maintained to 89 days, then gradually decreased (Fig. 1).

Characterization of antibodies:

The earliest detectable HI antibody at 7 days was in the first protein peak (Fractions 19 to 23) on gel filtration, indicating it to be of the 19S class (Fig. 2-A). Gel filtration of a 21 day’s serum, showed HI activity in the first peak (Fractions 20 to 24), where 19S γ-globulin is found, and HI activity in the second peak (Fractions 25 to 32), where 7S is located (Fig.
Transmission of IgG from Hen to Chicks

Fig. 1 Hemoagglutination inhibiting antibody responses in a domestic hen inoculated with a series of two subcutaneous injections of Japanese encephalitis virus. 
V: Viremia was recognized. ↑: Inoculation of J. E. virus.

Fig. 2-A
Fig. 2 Separation by gel filtration with Sephadex G-200 of domestic hen's serum obtained after a series of two subcutaneous injections of Japanese encephalitis virus. 
Fig. 2-A: Serum obtained 7 days after the first injection. Fig. 2-B: That 21 days after. Fig. 2-C: That 35 days later. Fig. 2-D: That 90 days later. Fig. 2-E: That 120 days later. Fig. 2-F: That 150 days later. 
Second inoculation was done 28 days after the first injection. Column was 45 cm in length and 2.6 cm in diameter, and the flow rate was 10 ml/hr. Eluates were collected in 3 ml amount.

2B). Gel filtration of 35 days serum (Fig. 2C) showed a slightly increased activity in the first peak and the bulk of activity in the second peak. At
89 days, similar results of that obtained 35 days were recognized (Fig. 2D). The detectable HI antibody at 120 days was found in the second protein peak (Fig. 2E). At 150 days, activity of the second peak was decreased (Fig. 2F).

Transmission of IgM or IgG from hen to chicks:

Hen, 10 month age, infected experimentally with JE virus, from 14 days prior to this experiment were bred with a non-immune roosters. Sera from the progenies hatched from the eggs born from 7 to 10 days after inoculation of each hen were taken. A 13-day postinoculation, serum of hen studied by gel filtration (Fig. 3A) showed HI antibody in two regions,
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Fig. 2-D

Fig. 2-E

Fig. 2-F

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Fig. 3 Separation by gel filtration with Sephadex G-200 of a serum in domestic hen obtained 13 days after the inoculation (Fig. 3-A) and a serum in progenies of hen, hatched from the eggs born 7 to 10 days after inoculation (Fig. 3-B).

The first and the second peaks. Gel filtration of chick serum (Fig. 3B) showed HI antibody in the second peak. Therefore, it was concluded that IgG antibody was transferred from hen to chicks, and IgM antibody was not.

The three group’s sera pooled from 3 chick sera in each showed 1:80 of titer in HI reaction before and after treatment of 2-ME, though serum of hen contained 2 ME sensitive antibody. This also showed that chick sera contained merely IgG antibody.
Transmission of IgG from Hen to Chicks

DISCUSSION

It is generally known that in infection of JE to swine and human being IgM is formed, which is later transformed to IgG within a month after infection (10, 11). However, there is no report concerning with domestic fowls. We also reported the appearance of 2 ME resistant antibody followed production of 2-ME sensitive antibody in the serum of hen, infected with JE naturally (6). In this experiment, transformation of IgM to IgG was recognized in the serum of experimentally infected hen with JE.

We described that 7 out of 19 domestic fowls proved HI positive reaction and 6 out of 7 positive fowls showed 2-ME sensitive reaction in summer (6). Considering this experiment, showing that IgM transforms to IgG in the experimentally infected hens, and above report the presence of primarily, naturally infected hen is confirmed. KITAOKA (1) described the presence of viremia in domestic fowls, inoculated JE virus and we also demonstrated the similar results in this experiment. Therefore, domestic fowls can be infection source of JE.

BUESCHER and SCHEIRER (5) described that HI and NT antibodies transferred from experimentally infected hens to chicks. And WATANABE (12) reported that S. pullorum antibody transferred from hen to chicks through egg yolk. We discussed about the problem whether IgG antibody was merely transferred from hen to chicks and IgM antibody was not. The mechanism of this phenomenon is now on investigation.

SUMMARY

Transformation of Japanese encephalitis antibody from IgM to IgG in the sera of the experimental infected chicks with Japanese encephalitis virus and transmission of IgM or IgG from hen to chicks were examined by the gel filtration on Sephadex G-200 column.

The following results were obtained.

1. Titer of hemoagglutination inhibiting antibody rose on seven days after inoculation of mouse brain homogenate infected with Japanese encephalitis, and that increased rapidly after the second inoculation of Japanese encephalitis. The maximum peak of antibody titer attained on 35 days after the first inoculation, on 7 days after the second inoculation and it maintained for a period of 2 months then decreased. Viremia was detected till 6 hours after the first inoculation.

2. IgM antibody by gel filtration appeared on 7 days after the first inoculation, kept on rising, reached the peak on 35 days after the first
inoculation, then decreased, and disappeared on 120 days. IgG antibody appeared about 2 weeks after the IgM antibody appearance, and the titer of IgG antibody became higher than that of IgM antibody on 35 days after the first inoculation, then decreased gradually, and showed 1:16 of titer of peak on 150 days by gel filtration.

3. We could obtain the chicks by fertilization from experimentally infected hen, having IgM and IgG of hemagglutination inhibiting antibody of Japanese encephalitis. And the localization of antibodies in the sera of its chicks was determined by Sephadex G-200 gel filtration. And IgG antibody was detected in chick serum, though IgM antibody was not detected by this method.

REFERENCE