Maternal antibody to infectious bronchitis virus: its role in protection against infection and development of active immunity to vaccine

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Abstract

Chicks hatched with high levels of maternal antibody had excellent protection (>95%) against infectious bronchitis virus (IBV) challenge at 1 day of age, but not at 7 days (<30%). This protection significantly ($P < 0.05$) correlated with levels of local respiratory antibody and not with serum antibody.

A high percentage of both maternal antibody-positive (Mab+) and maternal antibody-negative (Mab−) chicks failed to produce IBV antibody when vaccinated at 1 day of age by the intraocular route. In addition, Mab+ chickens had a weaker virus-neutralizing antibody response to a second IBV vaccination compared to Mab− birds ($P < 0.05$). Mab+ chicks experienced a more rapid decline ($P < 0.01$) in maternal antibody after 1-day-of-age vaccination compared to their unvaccinated counterparts.

A monoclonal antibody-based blocking ELISA that measured antibody levels specific to S1 glycoprotein of IBV correlated well with virus-neutralizing antibody titers. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Infectious bronchitis is a highly contagious and economically significant disease of chickens caused by infectious bronchitis virus (IBV), a coronavirus. Prophylactic

vaccination against IBV infection is practiced using both live and inactivated vaccines (Cavanagh and Naqi, 1997). Live IBV vaccines are administered to chickens through spray or drinking water, often as early as 1 day of age, whereas inactivated vaccines are injected subcutaneously or intramuscularly before the onset of the egg-laying cycle (Cavanagh and Naqi, 1997). IBV antibody can be detected in the serum and respiratory passages of vaccinated birds by various serological tests (Gelb, 1989).

IgG-class of antibody, passed from vaccinated hens via the yolk to the progeny, can be detected in serum and respiratory mucus of newly hatched chicks (Jungherr and Terrell, 1948; Hawkes et al., 1983). Such antibody has been shown to protect chicks against IBV challenge for 4 weeks depending on the methods used for challenge and subsequent evaluation of protection (Darbyshire and Peters, 1985; Mockett et al., 1987). Vaccination of 1-day-old commercial broiler chickens against IBV is widely performed. This practice raises two important concerns: (1) influence of maternal antibody (Mab) on the development of active immune response to the vaccine, and (2) effect of vaccine virus on the rate of depletion of Mab. Another basic concern is whether neonatal administration of IBV to chicks via spray or drinking water could induce an immunologic tolerance to one or more IBV antigens. The latter is a logical concern based on studies conducted in mammalian species where tolerance has been demonstrated to mucosally administered antigens (McGhee et al., 1992; van Wilsem et al., 1995; Galliaerde et al., 1995; Lamm, 1997; Weiner, 1997). In the present study, day-old vaccination did not induce ''tolerance'' to IBV, nevertheless, it was found to: (a) hasten depletion of Mab, and (b) interfere with antibody responses to both primary and secondary immunizations.

2. Materials and methods

2.1. Chickens

Maternal antibody-free (Mab−) White Leghorn-type chickens were acquired from flocks maintained by our department, whereas maternal antibody-positive (Mab+) chickens were obtained from a commercial hatchery. Different treatment groups were housed in separate isolation rooms and were subjected to similar husbandry practices.

2.2. Vaccine

A live commercially produced Massachusetts-serotype IBV vaccine was used. The vaccine was diluted as per the manufacturer's recommendations, and administered intraocularly. The vaccination schedule is outlined in Table 1.

2.3. Collection of blood and tracheal washes

These procedures have been described previously (Mockett et al., 1987; Thompson et al., 1997). Blood was collected either from the jugular vein or brachial vein, and

Table 1
Summary of Experimental design for Experiments 2 and 3

Experiment number	Total number of chickens used	Mab[a] status	Age at vaccination (days)	Number of chickens examined at age (days)			
				7	14	21	28
2	122	+	1	18	18	18	–
			1, 21	–	–	–	18
			Non-vac[b]	10	10	10	10
3	70	–	1	10	10	10	–
			1, 21	–	–	–	10
			Non-vac[b]	5	5	5	5

[a] Maternal antibody (Mab) status at 1 day of age.
[b] Additional ten 1-day-old chicks (not shown in the table) were examined for serum and respiratory antibody prior to vaccination.

chickens were subsequently euthanized by CO_2 asphyxiation. In Experiment 1, the respiratory tracts of chickens were washed as described by Thompson et al. (1997). Briefly, tracheae were exposed just below the glottis and cannulated. The respiratory system of each bird was flushed three times with a total of 10 ml of warm (37°C) PBS, and the three washes from each bird were pooled. Respiratory lavage fluids in Experiments 2 and 3 were collected as described by Mockett et al. (1987) with slight modification. In this case, tracheal segments were washed between a point immediately distal to the rima glottis and immediately proximal to the point where the trachea enters the thoracic cavity. Using a micropipett with disposable tips, 0.8 ml of PBS was used to flush the trachea three times with the same fluid. The collected lavage fluids were then passed through a fine mesh (60 μm) screen, and centrifuged. The supernatant fluids were removed and used for the assessment of IBV-specific local antibody. Sera as well as lavage fluids were heat inactivated (56°C for 30 min) before use in the virus neutralisation test described below.

2.4. ELISA

Two ELISA procedures were used. Total IBV antibodies were measured in individual sera and lavage fluids using a standard indirect ELISA (I-ELISA) (Karaca et al., 1990). On the other hand, determination of antibody specific to IBV spike (S1) glycoprotein were made using a monoclonal antibody-based blocking-ELISA (B-ELISA) (Karaca and Naqi, 1993). Positive ELISA reactions were determined as described previously (Karaca et al., 1990; Karaca and Naqi, 1993), and geometric mean titers (GMT) were calculated (Villegas and Purchase, 1989).

2.5. Virus-neutralization (VN) test

A beta procedure (constant virus against serially diluted serum) was used to assess VN antibody levels in serum and respiratory lavages. Beaudette strain (a Massachusetts

serotype) of IBV, which propagates well in a chicken hepatocellular carcinoma cell line (LMH cells), was used (gratefully received from Dr. B. Cowen, BIOMUNE, KS). Briefly, 2-fold dilutions of chicken serum (starting dilution $= 1 : 20$) and lavage fluids (starting dilution $= 1 : 2$) were mixed with approximately 100 tissue culture infective doses 50% ($TCID_{50}$) of the virus, and the mixture was incubated for 60 min at $37^{\circ}C$. Each serum-virus or lavage-virus mixture was applied to duplicate wells of 96-well tissue culture plates (Corning Glass Work, NY) in which LMH cell monolayers were grown in advance, followed by a 60-min incubation at $37^{\circ}C$, before cell monolayers were overlaid with William's medium (GIBCO BRL, Cat. no. 31500-028) containing 10% fetal bovine serum. After 72 h of incubation at $37^{\circ}C$ in a 5% CO_2 environment, the cells were fixed with an acetone:methanol (55:45) mixture (v/v) for 10 min, and stained for the presence of IBV by a monoclonal antibody-based immunoperoxidase (IP) method (Naqi, 1990). The reciprocal of the highest dilution of serum or lavage fluid that completely neutralized the virus was considered as the serum titer.

2.6. Experimental design

2.6.1. Experiment 1

This was conducted to address two objectives: (1) to monitor the decline of maternally-acquired IBV antibody in the serum and the respiratory system during early life, and (2) to study the efficacy of this antibody in protecting chicks against an intraocular challenge with IBV.

Two hundred, 1-day-old Mab+ commercial Leghorns were obtained from a commercial source. On days 1, 7, 10, 14, and 17, two groups of 20 chicks were removed, one group was bled for serum collection and then euthanized to obtain respiratory lavage fluids for Mab estimation, and other group was intraocularly challenged with approximately 10^5 embryo infective dose 50% (EID_{50}) Massachusetts-type IBV per bird. Four days after the challenge, the birds were killed and tracheae were collected for virus isolation in embryonic chicken eggs (Gelb, 1989). A group of 10 Mab− chicks was similarly challenged with IBV at each interval to serve as control.

2.6.2. Experiments 2 and 3

These were designed to assess the effects of day-old vaccination on: (1) depletion of maternal antibody in Mab+ chicks, and (2) development of primary and secondary immune responses in Mab+ and Mab− chicks. Antibodies in these experiments were measured against both, the whole virus and the S1 glycoprotein after primary and secondary vaccination.

The experimental design used for Experiments 2 and 3 is summarized in Table 1. Before assigning to different groups, 10 randomly selected chickens were bled and lavage fluids collected to assess serum and respiratory antibody levels against IBV and S1 glycoprotein. The chicks were then divided into three groups: a group vaccinated at day 1, another at days 1 and 21, and the 3rd maintained as unvaccinated control. The vaccine was applied intraocularly to individual chickens in all cases. After each vaccination, serum and tracheal lavage fluids were collected at weekly intervals as outlined in Table 1.

2.7. Stastistical analysis

Geometric mean titers were calculated using the standard procedures (Villegas and Purchase, 1989), whereas the mean titers for the treatment groups were compared using a Student's *t*-test as described earlier (Bishop, 1966).

3. Results

3.1. Experiment 1

Fig. 1 summarizes results of antibody measurement by I-ELISA against whole virus antigen and those of IBV challenge studies. At hatching, Mab+ chicks had significant levels of antibody in serum and the respiratory tract, as represented by the logarithm ($\log_{10}$) of geometric means, 5.2 log and 2.7 log, respectively. When challenged with IBV through the intraocular route at 1 day of age, these chicks had excellent protection with only 1 of 19 chicks yielding the challenge virus (>95% protection) 4 days post-challenge. At 7 days of age, while no remarkable change was observed in serum antibody titer, that in the respiratory system had dropped to approximately half (1.5 log) of the level recorded at day 1. This coincided with a drastic drop (<30%) in protection to virus challenge at day 7. During the remaining period (10–17 days), the serum-antibody levels declined gradually, remaining at an appreciable level until day 17 (>3.0 log), whereas, respiratory antibody levels fell below 1.0 log during the same period. There was no protection to IBV challenge on days 14 and 17. One hundred percent of the Mab− control chickens yielded virus at 4 days post-challenge at each of the 5 periods (data not shown).

3.2. Experiments 2 and 3

Fig. 2a illustrates serum-antibody levels (I-ELISA) of individual Mab+ birds after vaccination at 1 and 21 days of age. When I-ELISA was performed at 7 days of age, 5 of
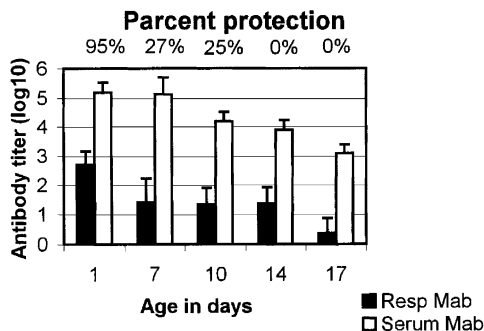

Fig. 1. Decline of maternal antibody (Mab) in the serum and respiratory system during the first 17 days post-hatching and its role in protection to IBV challenge (birds were challenged on 1, 7, 10, 14, 17 days of age and virus isolation was attempted 4 days post-challenge).
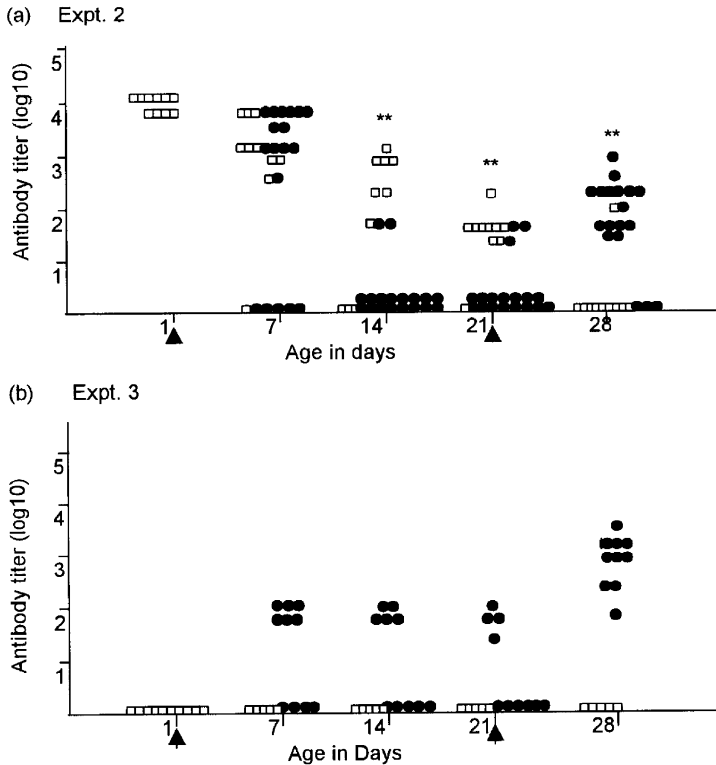
Fig. 2. (a) IBV antibody levels (I-ELISA) in the sera of individual maternal antibody-positive (Mab+) chicks; Titers of both vaccinated (●) and unvaccinated (□) birds are presented ([**]$P < 0.01$); (b) IBV antibody levels (I-ELISA) in the sera of individual maternal antibody-negative (Mab−) chicks. Titers of both vaccinated (●) and unvaccinated (□) birds are presented. The arrows indicate the time of vaccination.

the 18 vaccinated chickens and 1 of the 10 unvaccinated chickens had no detectable serum antibody. Nevertheless, the mean serum antibody levels of the two groups did not differ significantly ($P > 0.05$) at day 7. However, at days 14 and 21, such differences became highly significant ($P < 0.01$) as the majority of vaccinated birds (16/18 and 15/18, at 14 and 21 days, respectively) had no detectable serum antibody as compared to only 3/10 and 1/10 unvaccinated chickens at the respective days. However, secondary vaccination at 21 days did elicit a statistically significant ($P < 0.01$) antibody response in the majority (15/18) of Mab+ chickens when sera were examined 7 days after vaccination.

In contrast to Mab− chickens, the majority of Mab− chickens (6/10) developed IBV antibody 7 day after the day-old vaccination (Fig. 2b). Although, on days 14 and 21, only 5 of the 10, and 4 of the 10 chickens were antibody positive, secondary vaccination at 21 day of age resulted in antibody responses in all 10 birds examined 7 days later.

Sera from Experiments 2 and 3 were also assayed simultaneously by VN test and S1-specific B-ELISA described above (Fig. 3a and b). Fig. 3a indicates that Mab+ chickens
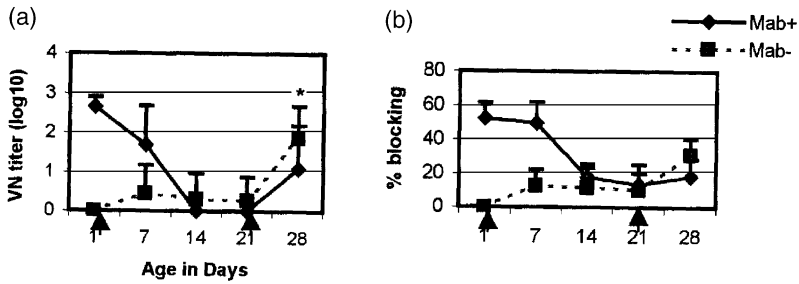
Fig. 3. Serum antibody response of maternal maternal antibody-positive (Mab+) and antibody-negative (Mab−) chicks to vaccination at 1 and 21 days of age measured by virus-neutralization (a) and S1-specific antibody-blocking ELISA (b) (*$P < 0.05$). The arrows indicate the time of IBV vaccination.
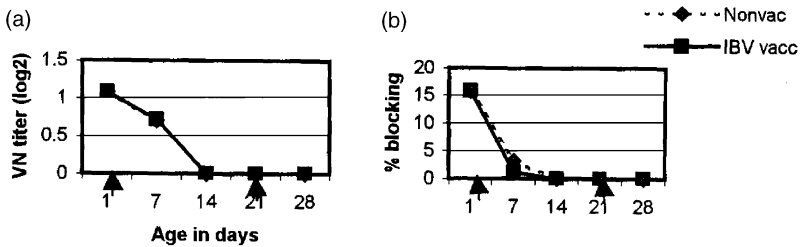

Fig. 4. IBV antibody in the tracheal lavage fluids of maternal antibody-positive (Mab+) chicks at 1 and 21 days of age measured by virus neutralization (VN) test (a) and S1-specific antibody-blocking ELISA (b). The arrows indicate the time of IBV vaccination. In figure 4a the hatched and the solid lines overlap.

vaccinated at 1 day of age had no detectable VN antibody in their sera when assayed at 14 and 21 days after vaccination. A similar conclusion can be drawn from the B-ELISA results (Fig. 3b) where a minimum of 30% blocking is considered necessary for the serum to have VN activity (unpublished data). It is also apparent that the day-old vaccination of Mab− chickens elicited only a feeble VN/B-ELISA response. Although, following the 2nd vaccination there was a rise in the antibody titer that was detectable by both assays, the anamnestic response, if any, was weaker in Mab+ chicks compared to their Mab− counterparts.

Antibody activity in tracheal lavage samples detected by I-ELISA and B-ELISA is presented in Fig. 4a and b, respectively. Overall, the antibody activity in trachea was low and no detectable response to vaccination was evident in either Mab− or Mab+ chickens, following primary or secondary vaccination.

4. Discussion

This study was to address: (a) whether the presence of Mab influences response of chicks to vaccines, (b) if 1-day-old vaccination affects the rate of depletion of Mab, and

(c) whether the mucosal administration of IBV vaccine at an early age induces antigen tolerance.

It is well known that commercially produced chicks carry significant levels of Mab at hatching (Jungherr and Terrell, 1948; Brambell, 1970). Nevertheless, there is uncertainty about the duration of protection from such antibody. Darbyshire and Peters (1985) vaccinated chicks with Holland 120 strain of IBV at 1 day of age, and at 4 weeks found the tracheae of vaccinated chickens were protected against an in vitro tracheal challenge by M41 strain of IBV. On the other hand, Mockett et al. (1987) reported, assuming protection on cumulative chick mortality during a 14-day post-challenge period, that the protection lasted only 7 days using an IBV (VF69–149 strain) plus *E. coli* challenge.

In the present study, high serum-antibody levels (>5.0 log) found in the sera of the Mab+ chicks remained unchanged during the first 2 weeks of life, whereas those in the respiratory system dropped by about 50% between days 1 and 7. Using a challenge procedure recommended by the United States Department of Agriculture (USDA), we found the protection to challenge drop from >95% at day 1 to <30% at day 7 (Fig. 1). Therefore, we concluded that the antibody in the respiratory tract and not that in the serum provided protection against the intraocular challenge.

It appears that vaccination of chicks at 1 day of age quickens the depletion rate of Mab in the circulation. This effect was significant ($P < 0.01$) on days 14 and 21 (Fig. 2a). Although the significance of this depletion is difficult to predict, if the serum antibody is to offer protection against spread of IBV from the respiratory tract to internal organs (reproductive tract and kidneys) via the blood stream, it is possible that lowering of circulating antibody levels could be consequential.

Vaccination of day-old chicks was generally ineffective in Mab+ chicks, and only marginally effective in Mab− birds (Fig. 2a and b, and Fig. 3a and b). This could be attributed to a number of factors, including immature status of the immune system at 1 day of age, partial neutralization of vaccine virus by Mab, and interference by maternal IgG in the development of active immunity. The latter has been convincingly demonstrated with regard to other vaccines (Subba Rao et al., 1978; Winterfield et al., 1980; Lucio and Hitchner, 1980; Naqi et al., 1983). Interestingly, development of VN antibody after the 2nd vaccination was also lower in Mab+ chicks compared to their Mab− counterparts (Fig. 3a,b).

Unfortunately, the tracheal lavage data for Experiments 2 and 3 are limited. This was due mainly to insufficient fluids obtained by washing the trachea alone (as opposed to washing the whole respiratory system as in Experiment 1). Nevertheless, the data indicate a trend that has been reported by us that (a) IBV does not elicit an appreciable VN antibody response in the respiratory tract, and (b) there appears to be absence of anamnestic response at the mucosal surface to IBV (Naqi and Jagne, 1999).

5. Conclusions

The short-term post-hatch protection to IBV challenge in Mab+ chicks was due to respiratory antibody and not the circulating antibody. Vaccination of Mab+ chicks at 1 day of age hastened the rate of Mab decline. That vaccination was also ineffective in

inducing adequate primary response, and, for yet unknown reasons, weakened the secondary immune response of the chicks. Data obtained in the present study are not adequate to allow the prediction that early-age mucosal administration of vaccine induces tolerance to individual IBV structural proteins.

Acknowledgements

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