FULL PAPER *Immunology*

Induction of Immune Suppression in the Chick by an Optimal Dose of an Immunizing Antigen in the Presence of its Specific Maternal Antibody

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ABSTRACT. Prolonged interference or suppression of maternal antibodies of the humoral immune response of newly hatched chicks to active immunization has been documented; however, the immunological mechanisms responsible for such suppression are still unclear. Laying hens were immunized with dinitrophenyl-keyhole limpet hemocyanin (DNP-KLH). Purified maternal anti-DNP or non-specific IgY antibodies were transferred by yolk sac inoculation to newly hatched chicks, and they were immunized with DNP-KLH or rabbit serum albumen (RSA) at 1 and 4 weeks of age. The concentrations of anti-DNP and anti-RSA antibodies in serum samples of these chicks were measured using an enzyme-linked immunosorbent assay (ELISA). The immune responses of the chicks that received a high dose of maternal anti-DNP antibodies and were immunized with an appropriate dose of DNP-KLH were suppressed. However, those of the chicks that received the same high dose of maternal non-specific IgY antibodies and were immunized with an appropriate dose of DNP-KLH and those of the chicks that received a high dose of maternal anti-DNP antibodies and were immunized with RSA were not suppressed. On the other hand, suppression of anti-DNP antibody production would not be induced if the chicks received a high dose of antigen specific maternal antibodies and were immunized with a high dose of the same antigen. These results revealed that the immune suppressive effect of maternal antibodies on the immune response of the newly hatched chicks was antigen specific and depended mainly on the ratio of antigen/maternal antibody at the time of immunization.

KEY WORDS: antigen specificity, chick, IgY, maternal antibody, suppression.

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Maternal derived antibodies provide immunological protection for newly hatched chicks against many environmental pathogens before active antibody production occurs. However, it has been found and reported that the presence of a high level of maternal antibodies can also interfere or suppress the ability of the young chick to actively respond to an early vaccination [6, 13, 21]. Prolonged interference or suppression of maternal antibodies of the humoral immune response of newly hatched chicks to active immunization has been documented [10]. It is also known that maternal IgG down-regulates neonatal Ig synthesis [20], perhaps by removing environmental antigens in the same manner as intravenous immunoglobulin treatment or through a direct effect on B cells. In humans and mice, the effect of maternal antibodies on early life responses has been widely studied. Most reports indicate that the presence of maternal antibodies further limits the intrinsic weakness of infant primary antibody responses to both natural infections and vaccinations [1]. The purpose of this study was to detect the antigen-specificity on the suppression by maternal antibodies and investigate the effect of the ratio of the antigen and maternal antibodies on the immune response of the newly hatched chick.

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MATERIALS AND METHODS

Antigen preparation: A classical hapten-carrier antigen, dinitrophenylated keyhole limpet hemocyanin (DNP-KLH), was used and prepared as previously described [11, 12]. Briefly, 200 mg of K_2CO_3 was dissolved in 6 m*l* of distilled water (DW) in a clean, dry and dark container, and then 200 mg of KLH (Calbiochem Behring Co., Germany) was added slowly during stirring on a magnetic stirrer. At the same time, 200 mg of 2, 4-dinitrobenzene sulfonic acid sodium salt (DNBS; Eastman Kodak Co., San Diego, U.S.A.) was dissolved in 4 m*l* of DW. DNBS solution was added into KLH solution. Subsequently, the mixture was stirred in a dark place at room temperature for about 18 to 24 hr, and then dialyzed against phosphate buffered saline (PBP) at 4°C several times until the optical density (OD) of the dialyzed buffer become zero at 360 nm against PBS. Finally, the mixture was sterilized by using a 0.45 μm filter. The protein The concentration of this antigen was determined by the OD value measured at 280 nm. The conjugation ratio of hapten with protein was determined as described previously [11, 12]. The final product was DNP_{32}-KLH. The antigen was then kept in a refrigerator at 4°C until use. Dinitrophenylated bovine serum albumin (DNP_{28}-BSA) was prepared in the same manner.

Animals: Partially inbred chickens (H-B15 white leghorn; Bu-1[a]) were used in this study. These chickens were bred in our animal facilities and were provided with food and chlorinated water *ad libitum* in accordance with the guidelines

for animal experiments at Hiroshima University. Eggs derived from the chickens were incubated and hatched in our own facilities. Chicks derived from non-immunized hens were ascertained to be free from maternal anti-DNP antibodies.

Immunization of laying hens: Ten laying hens (1–2 years old) were immunized by injection of DNP-KLH (1 mg per hen) emulsified in Freund's complete adjuvant (FCA) (Wako Pure Chemical Industries) into their peritoneal cavity. A second immunization was performed after 2 weeks, and the hens were then repeatedly immunized every 3 weeks using Freund's incomplete adjuvant (FIA; Wako Pure Chemical Industries, Osaka, Japan). Eggs derived from immunized laying hens were collected daily beginning one week after the second immunization and stored in a refrigerator at 4°C until use for extraction of IgY. The chicks, which were passively administered maternal anti-DNP antibodies were immunized two times with different doses of DNP-KLH (2 mg/kg of body weight [BW], 6 mg/kg of BW, and 20 mg/kg of BW) or RSA (2 mg/kg of BW) at 1 and 4 weeks of age. Chicks that received maternal non-specific IgY were immunized two times with DNP-KLH (2 mg/kg of BW) at 1 and 4 weeks of age. The first immunization was given into the peritoneal cavity with the antigen emulsified with FCA. The second immunization was given by the same manner but with FIA instead of FCA.

Purification of chicken IgY: Chicken IgY was extracted from the egg yolk by the water dilution method as described before [2]. Briefly, the egg yolk was separated from the white and diluted 1:10 in DW and its pH value was adjusted to 5.2. Then the solution was kept in a refrigerator at 4°C for at least 6 hr. Then, mixed gently and centrifuged (centrifugation for 15 min at 10,000 × g in a refrigerated centrifuge. The supernatant was decanted into a clean beaker, while stirring gently; ammonium sulfate (final percentage was 40%) was added gently and the mixing was continued for at least 30 min. The suspension was centrifuged for 15 min at 10,000 × g in a refrigerated centrifuge. Supernatant was discarded. Equal volume of PBS to the original volume of the egg yolk was added to the pellet and mixed gently until IgY pellet was completely dissolved. Then, IgY solution was dialyzed for 4–5 times against PBS until ammonium sulfate would be completely removed. The volume of purified IgY solution was measured after filtration with 0.45 μm filter. IgY purity was observed using sodium dodecyl sulphate-polyacrylamide gel electrophoresis, and simple band was observed (data not shown).

Affinity chromatography: Anti-DNP antibodies were measured by using a DNP-BSA conjugated Sepharose column. DNP-BSA was conjugated with swollen CNBr-activated Sepharose 4B beads (GE Healthcare, Sweden) in coupling buffer (bicarbonate buffer) (0.5 M NaCl, 0.1 M sodium bicarbonate, pH 8.3). Free-reacted sites on the beads were blocked with 1 M ethanol amine, pH 8.0, and the beads were washed with the coupling buffer and then the elution buffer (0.5 M NaCl, 0.2 M glycine-HCl, pH 2.5) four times before being washed with PBS and stored in a refrigerator at 4°C until use. The DNP-BSA column was washed gently with 5 m*l* of low pH buffer (0.5 M NaCl, 0.2 M glycine-HCl pH 2.5) and then gently washed with PBS (20 times the gel volume). After that, IgY solution was added several times to the Sepharose gel in the column. The filtrate was collected. The column was washed with PBS (20 times of the gel volume), and then 5 m*l* of the elution buffer (0.5 M DNP-EACA; Sigma Chemical Co., St. Louis, MO, U.S.A.) was added gently to the Sepharose gel in the column. The eluate, which contains anti-DNP antibodies, was collected, dialyzed against PBS to remove DNP-EACA until the optical density at 360 nm could not be detected, and then concentrated using a centrifugal filter device (Amicon ultra-15, ultracel 100k) (Millipore, U.S.A.). The optical density of the concentrated sample was measured at 280 and 360 nm for calculation of the actual amount of anti-DNP antibodies.

Inoculation of antibodies: Purified maternal anti-DNP antibody or non-specific IgY (200 μ*l* of 40 mg/m*l* in PBS) was injected into the yolk sacs of the newly hatched chicks (directly after hatching) as described previously [17] with some modifications. Briefly, the abdominal walls of the newly hatched chicks were sterilized with ethyl alcohol 70%; 1 m*l* syringes with 30-gauge needle (Becton Dickinson and Company, Franklin Lakes, NJ, U.S.A.) were used to inject antibodies into the yolk sac. The needle was inserted through the skin and into the yolk sac immediately posterior to the umbilicus where the sac closes to the abdominal wall.

Collection of blood: Blood samples were collected every week from each chick from the wing vein using a 1 m*l* syringe with 27G needle and stored at 4°C for 1 to 2 hr. Serum was separated from clotted blood by centrifugation at 10,000 × g for 5 min and stored at –80°C until use.

Enzyme-linked immunosorbent assay (ELISA): The concentrations of anti-DNP and anti-RSA antibodies were measured by ELISA as previously described [31]. Briefly, ELISA plates (Nunc, Roskilde, Denmark) were coated overnight with 55 μ*l* of DNP-BSA solution (50 μg/m*l*) or RSA solution (50 μg/m*l*) and then subjected to a 2 hr blocking period at 37°C with 350 μ*l* of 25% Block Ace (Dainippon Sumitomo Pharma, Co., Ltd., Osaka, Japan) in PBS. Four fold serum dilutions were then added, and the plates were incubated for 1 hr at 37°C. Each plate contained negative and positive control sample, especially in the case of measuring the concentration of anti-DNP antibodies. Following incubation, the plates were washed five times with PBS-Tween and then treated with diluted HRP-labeled goat anti-chicken IgY heavy and light chain (Bethyl Inc., Montgomery, TX, USA; 1/2,000, diluted in 10% Block Ace in PBS) for 1 hr. The plates were then washed five times with PBS-Tween, the substrate solution was added to the plate, the plate was left for 10–20 min in the dark place until it turned yellow and, the reaction was stopped using 2N H_2SO_4. Finally the optical density was measured at 490 nm with a micro plate reader (Model 680, Bio-Rad Japan, Tokyo, Japan). Each plate containing the dilution buffer only instead of a sample was considered a negative control, and

the standard purified anti-DNP antibodies (1 mg/m*l*) were considered the positive control. The concentration of serum anti-DNP antibody was measured after conversion of ELISA data into mg/m*l* by using a standard anti-DNP antibody sample with a known concentration. The levels of anti-RSA antibody were detected by measuring the half of plateau dilution units.

Statistical analysis: The mean of the concentration of anti-DNP antibodies and units of anti-RSA antibodies in the newly hatched chick's sera were compared using the Student's *t*-test. All values were expressed as means ± standard deviation and were considered to be significant at $p<0.005$.

RESULTS

Our study was divided into three experiments. In the first experiment, the concentration of anti-DNP antibody in the serum samples of the newly hatched chicks that received high levels of different maternal antibodies (anti-DNP antibody or antigen non-specific maternal IgY) on the day of hatching and were then immunized with DNP-KLH at 1 and 4 weeks of age, was measured. Eleven newly hatched chicks derived from non-immunized laying hens were divided into three groups. The first group consisted of four chicks and was immunized with DNP-KLH at 1 and 4 weeks of age. The concentration of anti-DNP antibodies (mg/m*l*) in the serum was measured in their serum samples using ELISA as mentioned previously. Their means are indicated as with open circles, with SD, in Fig. 1. The concentration of anti-DNP antibodies gradually increased after the first immunization, reached the maximum level (0.093 ± 0.018 mg/m*l*) at one week after the second immunization and decreased (0.050 ± 0.042 mg/m*l*) at two weeks after the second immunization. This group of chicks was considered to be a positive control group. The second group consisted of three chicks that received 8 mg of antigen non-specific maternal IgY per chick by yolk sac inoculation directly after hatching and were then immunized with DNP-KLH at 1 and 4 weeks of age. Their means are indicated with open triangles, with SD, in Fig. 1. The concentrations of anti-DNP antibodies of these chicks were not significantly different from those of the positive control group. The third group consisted of four chicks that received 8 mg of anti-DNP antibodies (antigen-specific maternal IgY) per chick by yolk sac inoculation directly after hatching and were then immunized with DNP-KLH at 1 and 4 weeks of age. Their means are indicated with closed circles, with SD, in Fig. 1. The concentrations of anti-DNP antibodies of these chicks were significantly lower than those of the positive control group and those that received an injection of normal IgY beginning the first week after the second immunization ($p<0.005$).

The 2nd experiment was carried out with the aim of measuring the concentration of anti-RSA in the serum samples of the newly hatched chicks that received a high dose of maternal anti-DNP antibodies on the day of hatching and were then immunized with RSA at 1 and 4 weeks of age.

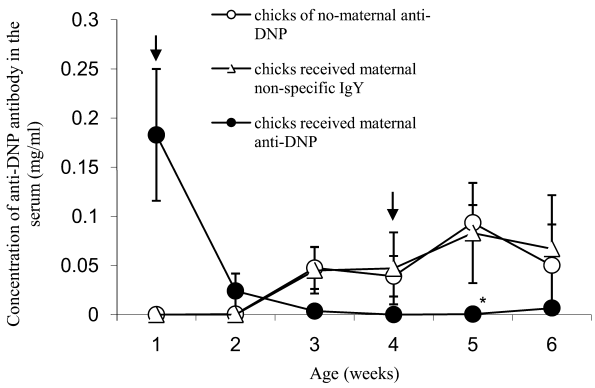

Fig. 1. Effect of maternal anti-DNP antibodies on the immune response to DNP. The concentrations of anti-DNP antibodies in the serum of newly hatched chicks that received an injection of 8 mg of anti-DNP IgY (closed circle), 8 mg of non-specific IgY (open triangle) or no additional IgY (open circle) into the yolk sac directly after hatching and were then immunized with DNP-KLH at 1 and 4 weeks of age were measured. *, $p<0.005$. The arrows indicate the time of immunization.

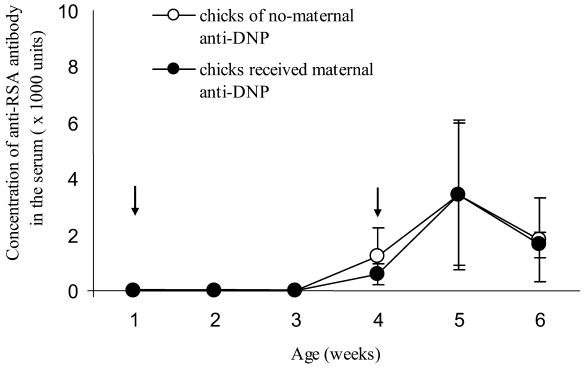

Fig. 2. Effect of maternal anti-DNP antibodies on the immune response to RSA. The concentrations of anti-RSA antibodies in the serum of newly hatched chicks that received an injection of 8 mg maternal anti-DNP IgY (closed circle) or no additional IgY (open circle) into the yolk sac directly after hatching and were then immunized with RSA at 1 and 4 weeks of age were measured. The arrows indicate the time of immunization.

Nine newly hatched chicks derived from non-immunized hens were divided into 2 groups. The 1st group was consisted of 5 chicks immunized with RSA at 1 and 4 weeks of age. The concentration of anti-RSA antibodies in the serum samples was measured by ELISA as previously mentioned. Their means are indicated with open circles, with SD, in Fig. 2. The concentration of anti-RSA antibodies gradually increased after the first immunization, reached the maximum level at 1 week after the second immunization and then decreased at 2 weeks after the 2nd immunization. This group of chicks was considered to be a control group. The 2nd group consisted of 4 chicks that received 8 mg of anti-DNP antibodies per chick by yolk sac inoculation directly after hatching and were then immunized with RSA at 1 and

4 weeks of age. Their means are indicated with closed circles, with SD, in Fig. 2. The concentration of anti-RSA antibodies in these chicks was normal, and there was no significant difference between the immune response of this group and that of the control group.

In the third experiment, the effect of antigen dose on chick immune response was investigated; the concentration of anti-DNP antibodies in the serum samples of the newly hatched chicks that received a high dose of maternal anti-DNP antibodies on the day of hatching and were then immunized with different doses of DNP-KLH at 1 and 4 weeks of age was measured. Twenty newly hatched chicks derived from non-immunized hens were divided into three groups. The 1st group consisted of 6 chicks (three of them received 8 mg of anti-DNP antibodies) immunized with DNP-KLH (2 mg/kg of BW) at 1 and 4 weeks of age. The 2nd group consisted of 7 chicks (three of them received 8 mg of anti-DNP antibodies) immunized with DNP-KLH (6 mg/kg of BW) at 1 and 4 weeks of age. The 3rd group consisted of 7 chicks (three of them received 8 mg anti-DNP antibodies) immunized with DNP-KLH (20 mg/kg of BW) at 1 and 4 weeks of age. The concentrations of anti-DNP antibodies of these chicks were measured by ELISA as mentioned before. Open circles, with SD, and close circles, with SD represent the data for chicks without maternal antibodies and the data for chicks with maternal anti-DNP antibodies, respectively, in Fig. 3. There was no significant difference between the humoral immune responses of the normal chicks and those of the chicks that received maternal anti-DNP antibodies and were then immunized with 6 mg or with 20 mg of DNP-KLH (Fig. 3B and 3C). Significant suppression was only observed in the case of immunization with 2 mg of DNP-KLH (especially at the 2nd and 3rd week after the 1st immunization and at the 1st week after the 2nd immunization, $p<0.005$, Fig. 3A).

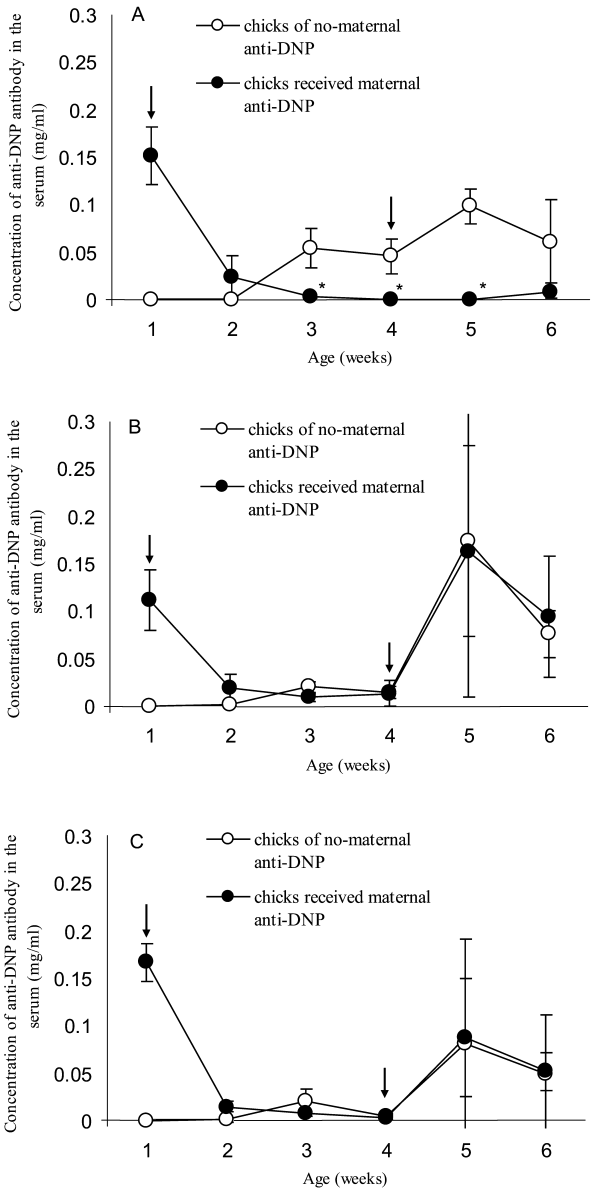

Fig. 3. Effect of the antigen doses for the suppression by maternal anti-DNP antibodies. The concentrations of anti-DNP antibodies in the serum of newly hatched chicks that received an injection of 8 mg of anti-DNP IgY (close circle) or no additional IgY (open circle) into the yolk sac directly after hatching and were then immunized with different doses of DNP-KLH at 1 and 4 weeks of age were measured. (A): Immune response of the chicks immunized with DNP-KLH (2 mg/kg of BW). (B): Immune response of the chicks immunized with DNP-KLH (6 mg/kg of BW). (C): Immune response of the chicks immunized with DNP-KLH (20 mg/kg of BW). *; $p<0.005$. The arrows indicate the time of immunization.

DISCUSSION

The large poultry population necessitates the use of early vaccination, as it is an effective tool to prevent and/or decrease the adverse effects of specific diseases, but the response to vaccination is affected by immaturity of the immune system and by the presence of maternal immunity [6]. The significance of passively transferred maternal antibodies lies first in the fact that they provide early protection to newly hatched chicks and, 2nd, in the fact that as a result of immunoregulatory mechanisms, they can interfere with or suppress immune responses to active immunizations [23, 26, 29]. The inhibitory effect of the passively derived maternal antibodies on the humoral immune response of neonates has been extensively reported [3, 4, 5, 7–9, 16, 25, 30]. The effect of such antibodies on the immune systems of newly hatched chicks remains an important area of investigation. This study was carried out with the aim of investigating and evaluating the antigen specificity of maternal antibodies and its effect on the immune response of newly hatched chicks that were immunized with different antigenic doses. Our study was divided into 3 experiments. The 1st experiment was carried out to detect the effect of a high level of different types of maternal antibodies (antigen specific and antigen non-specific antibodies) on the immune

response of newly hatched chicks that were immunized with the same antigen. The 2nd experiment was carried out to detect the effect of a high level of antigen specific maternal antibody (anti-DNP) on the immune response of newly hatched chicks that were immunized with a different antigen (RSA). The 3rd experiment was carried out to detect the effect of a high level of antigen specific maternal antibody (anti-DNP) on the immune response of newly hatched chicks that were immunized with different doses of an immunizing antigen (DNP-KLH).

The 1st experiment revealed that there was no suppression if the chick had maternal antibody which do not contain enough amounts of anti-DNP antibodies then immunized with DNP at 1 and 4 weeks. The 2nd experiment revealed that there was no suppression if the immunizing antigen was different with the specificity of maternal antibody. The 3rd experiment revealed that suppression of the humoral immune response was only observed when the chicks received 8 mg of anti-DNP antibodies and were immunized with 2 mg of DNP-KLH. Chicks immunized with a high dose of DNP-KLH could show normal immune response against DNP, even if they previously received 8 mg of maternal anti-DNP antibodies.

In conclusion, only optimum amounts of an antigen with its suitable specific maternal antibody can suppress the humoral immune response in the chick. It is not yet known why suppression would be induced in the chick. Several studies have documented the inhibitory effect of passive antibodies on the immune response to active vaccinations, but the mechanisms of this inhibition remain unclear. High concentrations of IgG can completely inhibit the protection provided by vaccination [14], probably by direct competition for the antigen with the cellular receptors and effectively blocking priming and indirect induction and maintenance of specific suppressor T cells, which act to inhibit the generation of memory T helper cells involved in IgG production [15]. In general, IgG antibodies suppress the production of both IgM and IgG antibodies, whereas, specific antibodies tend to suppress a specific immune response better than non-specific antibodies, as seen in the method employed to prevent hemolytic disease of the newborn in human [27]. This is thought to be mainly due to the negative feedback mechanism produced by total circulating maternal IgG [22, 28] or may be due to masking of antigenic epitopes [18, 19, 24], thereby removing the stimulus for proliferation of antibody producing cells. Our data in this paper indicates that the suppressive effect of antigen specific maternal antibodies on the immune response of newly hatched chicks depends mainly on the antigen/antibody ratio at the time of immunization. Investigation of the mechanism of this suppression must be continued in the future. We indicated in this paper that suppression was induced in the chicks by injection of maternal anti-DNP antibody and immunization with an optimal dose of DNP antigen. However, whether or not this suppression of humoral immunity in the chick is specific for only anti-DNP response is not yet known. We intend to address this in a subsequent article.

REFERENCES

1. Adkins, B., Leclerc, C. and Marshall-Clarke, S. 2004. Neonatal adaptive immunity comes of age. *Nat. Rev. Immunol*. **4**: 553–564.
2. Akita, E. M. and Nakai, S. 1993. Comparison of four purification methods for the production of immunoglobulin from eggs laid by hens immunized with an enterotoxigenic E.coli strain. *J. Immunol. Methods* **160**: 207–262.
3. Blomqvis, G., Lövgren-Bengtsson, K. and Morein, B. 2003. Influence of maternal immunity on antibody and T-cell response in mice. *Vaccine* **21**: 2022–2031.
4. Bjorkholm, B., Granstrom, M., Taranger, J., Wahl, M. and Hagberg, L. 1995. Influence of high titers of maternal antibody on the serologic response of infants to diphtheria vaccination at three, five and twelve months of age. *Pediatr. Infect. Dis. J.* **14**: 846–850.
5. Brugha, R., Ramsay, M., Forsey, T. and Brown, D. 1996. A study of maternally derived measles antibody in infants born to naturally infected and vaccinated women. *Epidemiol. Infect.* **117**: 519–524.
6. Chu, H. P. and Rizk, J. 1975. The effect of maternal immunity, age at vaccination and doses or live vaccines on immune response to Newcastle disease. *Dev. Biol. Stand.* **28**: 451–463.
7. Claire, A. S., Christy, B., Xanvier, M., Christian, B., Monika, B., Marco, C., Jiri, K. and Paul, H. L. 1998. Influence of maternal antibodies on vaccine response: inhibition of antibody but not T cell responses allows successful early prime-boost strategies in mice. *Eur. J. Immunol.* **28**: 4138–4148.
8. Claire, A. S., Marco, C., Christian, B., Christy, B., Monika, B., Chantal, T., Jiri, K. and Paul, H. L. 1998. Determinants of infant responses to vaccines in presence of maternal antibodies. *Vaccine* **16**: 1409–1414.
9. Claire, A. S. 2001. Neonatal and early life vaccinology. *Vaccine* **19**: 3331–3346.
10. Elazab, M. F. A., Fukushima, Y., Horiuchi, H., Matsuda, H. and Furusawa, S. 2008. Prolonged suppression of chick humoral immune response by antigen specific maternal antibody. *J. Vet. Med. Sci.* **71**: 417–424.
11. Furusawa, S., Okitsu-Negishi, S., Yoshino, K., Mizoguchi, M. and Noguchi, Y. 1987. Anti-idiotypic antibody as a mirror image of the paratope of the original antibody. *Int. Arch. Allergy Appl. Immunol*. **84**: 263–270.
12. Good, A. H., Wofsy, L., Henry, C. and Kimura, J. 1980. Preparation of hapten-modified protein antigens. pp. 343–350. *In*: Selected Methods in Cellular Immunology (Mishel, B.B. and Shiigi, S.M. eds.), Freeman, San Francisco.
13. Goddard, R. D., Wyeth, P. J. and Varney, W. C. 1994. Vaccination of commercial layer chicks against infectious bursal disease with maternally derived antibodies. *Vet. Rec.* **135**: 273–274.
14. Harte, P. G., De Souza, J. B. and Playfair, J. H. L. 1982. Failure of malaria vaccination in mice born to immune mothers. *Clin. Exp. Immunol.* **49**: 509–516.
15. Harte, P.G. and Playfair, J. H. 1983. Failure of malaria vaccination in mice born to immune mothers. II. Induction of specific suppressor cells by maternal IgG. *Clin. Exp. Immunol.* **51**: 157–164.
16. Hayley, A. G., Yvonne, M., Linda, L. Y., Judy, B., Susette, A., Mary, M. R., Ross, D. and Ann, M. A. 1999. IL-12, IFN-γ, and T cell proliferation to measles in immunized infants. *J. Immu-*

nol. **162:** 5569–5575.
17. Henry, D. S., Max, B. and Zhixun, X. 1992. Simulation of maternal immunity by inoculation of immune yolk preparations into the yolk sac of 1-day-old chickens. *Avian Dis.* **36**: 1048–1051.
18. Heyman, B. 1999. Antibody feedback suppression: towards a unifying concept? *Immunol. Lett.* **68**: 41–45.
19. Heyman, B. 2000. Regulation of antibody responses via antibodies, complement, and Fc receptors. *Annu. Rev. Immunol.* **18**: 709–737.
20. Klobasa, F., Werhahn, E. and Butler, J. E. 1981. Regulation of humoral immunity in the piglet by immunoglobulins of maternal origin. *Res. Vet. Sci.* **31**: 195–206.
21. Kumar, K., Singh, K. C. P. and Prasad, C. B. 2000. Immune responses to intermediate strain IBD vaccine at different levels of maternal antibody in broiler chickens. *Trop. Anim. Health Prod.* 32**:** 357–360.
22. Margulies, M., Voto, L. S., Mathet, E. and Margulies, M. 1991. High-dose intravenous IgG for the treatment of severe rhesus alloimmunization. *Vox Sang.* **61**: 181–189.
23. Mazariegos, L. A., Lukert, P. D. and Brown, J. 1990. Pathogenicity and immunosuppressive properties of infectious bursal disease 'intermediate'strains. *Avian Dis.* **34**: 203–208.
24. Mikeal, C. I. K., Sara, W., Teresita, D. S., Susanne, G. and Birgitta, H. 1998. Efficient IgG-mediated suppression of primary antibody responses in Fcγ receptor-deficient mice. *Proc. Natl. Acad. Sci. U.S.A.* **96:** 2244–2249.
25. Morein, B., Abusugra, I. and Blomqvist, G. 2002. Immunity in neonates. *Vet. Immunol. immunopathol.* **87**: 207–213.
26. Tizard, I.R. 2002. The avian antibody response. *Semin. Avian Exotic. Pet. Med.* **11**: 2–14.
27. Tizard, I.R. 2004. Immunity in the fetus and newborn. pp. 207–219. *In*: Veterinary Immunology-an Introduction (Tizard, I.R. ed.), Saunders, Philadelphia.
28. Urbaniak, S. J., Duncan, J. I., Armstrong-Fisher, S. S., Abramovich, D. R. and Page, K. R. 1997. Transfer of anti-D antibodies across the isolated perfused human placental lobule and inhibition by high-dose intravenous immunoglobulin: a possible mechanism of action. *Br. J. Hematol.* **96**: 186–193.
29. Wyeth, P. J. and Chettle, N. J. 1990. Use of infectious bursal disease vaccines in chicks with maternally derived antibodies. *Vet. Rec.* **126**: 577–578.
30. Xiang, Z. Q. and Ertl, H. C. J. 1992. Transfer of maternal antibodies results in inhibition of specific immune responses in the offspring. *Virus Res.* **24**: 297–314.
31. Yasuda, M., Furusawa, S., Satoh, A., Taura, Y., Nakama, S., Yoshihara, K. and Hirota, Y. 1995. Effects of 6-hydroxydopamine on the antibody response to the hapten dinitrophenyl in the chicken. *J. Vet. Med. Sci.* **57**: 1073–1075.