Efficacy of Subcutaneous Application of Live Infectious Bursal Disease Vaccine in Young Chickens with Maternally Derived Antibody

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ABSTRACT. The infectivity of neutralized IBDV by normal chicken serum (NCS) was detected in day-old and 3-week-old chicken spleen adherent (CSA) cells, and that of neutralized IBDV by maternal antibody (MN-Ab) was detected in 3-week-old CSA cells. Moreover, CSA cells from day-old chickens had complement receptor (CR), and CSA cells from 1-week-old had both CR and Fc receptor (FcR). However, the infectivity of neutralized IBDV by MN-Ab was confirmed on CSA cells which were blocked for FcR on CSA cells by heat-aggregated NCS (56°C, 60 min). These results indicated that infection of neutralized IBDV by NCS on CSA cells occurred via CR, and neutralized IBDV by MN-Ab was infected via FcR. In day-old specific pathogen free (SPF) chickens, the antibody level in NCS treated and non-treated IBD live vaccine subcutaneously inoculated groups was higher than the levels in the MN-Ab-treated IBDV inoculated group, and detected until 28 days old. Moreover, subcutaneously inoculated chickens were protected against the challenge of wild IBDV at 21 days old, whereas subcutaneously inoculated chickens were infected with MN-Ab-treated IBD live vaccine. In commercial layers which had MN-Ab, antibody levels of subcutaneously vaccinated group were higher than both the non-vaccinated and orally vaccinated groups, and virus isolation and viral antigen were positive with high detection rates on peripheral lymphocytes of each subcutaneously vaccinated group of SPF and commercial chickens. There were no distinct pathological changes, no decrease in complement activity measured via the alternative pathway, and no secondary antibody response of NCS-treated and/or non-treated IBDV subcutaneously vaccinated SPF chickens and commercial layers, after challenge. These results suggest that the subcutaneous application of live vaccine to day-old chickens has a more protective effect against a virulent IBDV exposure at 21 days old than the oral application.—**KEY WORDS:** complement receptor, IBDV, maternal antibody, subcutaneous vaccination.

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Infectious bursal disease virus (IBDV) is a non-enveloped double stranded RNA virus of the *Birnaviridae* family [3]. The main target organ is the bursa of Fabricius and target cells are IgM bearing B cells [9], monocyte cells [4, 11] and chicken spleen adherent (CSA) cells [12, 13]. Virulent IBDV can induce an immunosuppression [8, 10]. Recent outbreaks of infectious bursal disease (IBD) in commercial flocks were associated with high mortality [2, 5, 23–25].

Among the many studies on live IBD vaccination [14, 15], there are no reports on the successful immunization of young chickens with high levels of maternal antibody (MN-Ab).

It is supposed that during the reduction in the concentration of antibody with age, IBD vaccine virus will be in competition with the field virus. In practice, the field virus is supposed to be able to invade birds before vaccine virus, since MN-Ab would inhibit the latter [14, 16].

However, we previously reported that attenuated IBDV neutralized by complement or/and MN-Ab was still infective via complement receptor(s) (CR) or/and Fc receptor (FcR) on immune cells [12, 13]. This attenuated IBDV is also infective in day-old chickens in the presence of MN-Ab. Similarly, some viruses can infect via the CR or/and FcR [1, 6].

In this paper, we report the infectivity of neutralized

IBDV in CSA cells and its relevance to CR or/and FcR, and the effect of the live vaccine when applied subcutaneously in one-day-old chickens with MN-Ab.

MATERIALS AND METHODS

Cells: Primary monolayer cultures of chicken embryo fibroblast (CEF) prepared from 10- to 11-day-old specific pathogen free (SPF) chicken embryos (SPAFAS, Co., Ltd., Norwich, CT) were used for virus titration and virus isolation. CEFs in Eagle's minimum essential medium (MEM) with 5% heat-inactivated (56°C, 30 min) fetal calf serum (FCS) were cultured in plastic plates or dishes (Falcon 3002, 3008, Becton Oxnard, CA). CSA cells [12, 13] were obtained from day-old, 1-, 2- and 3-week-old SPF chickens, and were cultivated in RPMI 1640 medium with 10% heat-inactivated FCS on plastic dishes.

Normal chicken serum: Normal chicken serum (NCS) was prepared from 4- to 7-week-old SPF chickens, pooled and stored at –80°C until use as chicken complement. In a neutralization test, no specific antibody to IBDV was detected in NCS. Complement activities of both classical (CCP) and alternative (ACP) pathways were determined as described previously [17], and 50% hemolytic activity (CH₅₀) at 1:24 to 1:32 for the former and 1:20 to 1:24 for the latter with NCS was obtained. NCS and heated NCS

which was treated by heating (56°C for 30 min) [17] were used for *in vitro* study.

Antiserum: Anti-IBDV serum was obtained from day-old commercial layers (as MN-Ab) and heat-inactivated (56°C, 30 min) before use. The titer of the antiserum was measured by the 50% plaque reduction method and was determined at 4,500 units. For the preparation of anti-sheep erythrocyte chicken serum (AECS), 4-week-old SPF chickens were immunized twice with sheep erythrocytes, bled by heart puncture and the hemoagglutination (HA) titer was determined at 128 units, and used for the rosette formation test on day-old, 1-, 2- and 3-week-old CSA cells.

Examination of virus infectivity on heat-aggregated NCS treated CSA cells: The infectivity of K strain of live IBD vaccine (the Chemo-Sero-Therapeutic Research Institute, Kumamoto) [22] treated with MN-Ab, NCS and heated NCS was examined by means of the infectivity test involving CSA cells treated with heat-aggregated NCS (56°C, 60 min) [1]. The stock virus with $10^{3.0}$ TCID₅₀ per ml was incubated at 37°C for 1 hr with NCS, MN-Ab and heated NCS, and the virus inoculum was adjusted to contain 10 TCID₅₀ per ml of preincubation titer. By implementing this minimal dose, the NCS- and MN-Ab-treated virus solution was found to contain no infectious virus. One ml of virus solution was then inoculated into each of 4 plastic dishes seeded with day-old and 3-week-old CSA cells which were pretreated with heat-aggregated NCS or not. After incubation at 37°C for 4 days, the infectivity titers of the culture supernatants were measured in CEFs [12, 13].

Examination for complement receptor(s): To determine the existence of CR on CSA cells, zymosan (Sigma Chemical Co., St Louis, MO) treated with NCS and fluorescent iso-thiocyanate (FITC: Organo Technika Co., West Chester, PA) was prepared. Briefly, 2 mg of zymosan was washed 3 times, incubated with 2 ml of NCS at 37°C for 30 min, and labeled with FITC. The FITC labeled and NCS treated zymosan was washed 3 times with phosphate buffer saline (PBS). The phagocytic activity of day-old, 1-, 2- and 3-week-old CSA cells via CR was observed under a fluorescent microscope.

Chickens: Fertile SPF chicken eggs and layer-eggs from a commercial parent layer-flock (Dekalb), which had been vaccinated with live and killed IBD vaccine, were hatched in this laboratory and raised in an isolator system. The one-day-old layer chickens had neutralizing antibody titers over 1: 1,000 measured in a 50% plaque reduction test on CEF cultures.

Firstly, to investigate the infectivity of live IBD vaccine treated with NCS and MN-Ab to day-old SPF chickens and the effect of protection, SPF chickens were divided into four groups of 35 birds per group as follows: subcutaneous vaccination with non-treated IBD live vaccine and challenge (A); subcutaneous vaccination with NCS treated live IBD vaccine and challenge (B); subcutaneous vaccination with MN-Ab treated live IBD vaccine and challenge (C); no vaccination and no chal-

lenge (D). Secondly, to examine the efficacy of subcutaneous application of IBD live vaccine in day-old commercial layers, chickens were divided into four groups of 35 birds per group as follows: subcutaneous vaccination and challenge (E); oral vaccination and challenge (F); challenge only (G); no vaccination and no challenge (H).

Vaccination and challenge of IBDV: A live IBD vaccine virus, strain K, was adjusted to $10^{5.3}$ TCID₅₀ per ml and used for on *in vitro* study. In the *in vivo* study, K strain was adjusted to $10^{4.5}$ TCID₅₀ per bird and administered subcutaneously in the neck or orally in day-old chickens. For both B and C group chickens, K strain was incubated with NCS or MN-Ab (adjusted to 200 units) at 37°C for 30 min [12, 13], and both treated virus titers were decreased at $10^{3.0}$ TCID₅₀ per bird. This live vaccine strain was an attenuated IBDV and induced no pathological changes in SPF chickens [22] when given either subcutaneously or by oral inoculation. A challenge strain of IBDV, GBF-1 [7], was adjusted to 10% suspension of bursa of Fabricius upon harvest 4 days after infection of 6-week-old chickens, and used for oral challenge (0.2 ml per bird) of 3-week-old chickens.

IBDV antibody measurement: Serum was collected by wing vein puncture from 5 birds per group at 0, 7, 14, 21, 25, 28 and 35 days of age and heat-inactivated at 56°C for 30 min. The serum sample was diluted five hundred fold and antibody to IBDV was measured by a commercial enzyme linked immunosorbent assay (ELISA: IDEXX Co., Portland, ME) [15, 21]. The antibody response was calculated as sample/positive (s/p) ratio of the serum.

Examination for viral antigen: The bursae of Fabricius and peripheral white blood cells obtained from 5 birds per group at 3, 5, 7, 14 and 21 days post inoculation (pi) were examined for the presence of viral antigen by a direct fluorescent antibody test (FAT).

For the FAT, bursa of Fabricius was frozen with acetone in dry ice and sectioned in a cryostat at 6 to 9 µm on glass slides. The peripheral lymphocytes were prepared from peripheral blood smeared on glass slides for 2 days and frozen with acetone in dry ice. Frozen smears or sections were stained with FITC conjugated antibody (IgG) to IBDV. Antibodies were precipitated from SPF chicken serum that had been infected with GBF-1, and were purified by Sephadex G-150 (Pharmacia, LKB, Uppsala, Sweden). Conjugation with FITC was performed as previously described [19].

Virus isolation test: For virus isolation, bursae (0.2 mg) were homogenized, whereas peripheral lymphocytes were obtained from peripheral blood (1 ml at 3-day-old, 2 ml after 5-day-old) by separation with Lymphocyte-Separator Solution (Sigma Chemical Co., St. Louis, MO), centrifuged at 1,000 rpm for 5 min, and washed 3 times with Eagle's MEM [13]. These samples were inoculated onto CEF cultures that were incubated at 37°C for 7 days and inspected daily for cytopathic effect (CPE).

Clinical and histopathological observation: Birds were observed clinically until 35 days old (2 weeks after challenge). Then tissues of the bursae of Fabricius from

Table 1. Virus growth of IBDV treated with MN-Ab or NCS in heat-aggregated NCS^{a)} treated and non-treated CSA cells

Age of chicken for CSA cells	CSA cells treated with	Virus treatment with		
		NCS	MN-Ab	Heated NCS
Day old	Non	2.0 ^{b)}	<1.5	4.0
Day old	Heat-aggregated NCS	2.0	<1.5	4.0
3 weeks old	Non	2.5	2.0	4.0
3 weeks old	Heat-aggregated NCS	2.25	<1.5	4.5

a) Heat-aggregated NCS (56°C, 60 min) was used for blocking Fc receptors on CSA cells.

b) Virus titer (TCID₅₀ per ml) of the culture supernatant of CSA cells.

five birds per group were collected, and the weight of bursae and body weight of each bird were measured to calculate the bursa/body weight ratio. Samples of bursa were fixed in 10% neutral buffered formalin and paraffin embedded sections were cut and stained with haematoxylin and eosin.

RESULTS

Infectivity of IBDV via the FcR and CR on CSA cells:

Table 1 shows the infectivity of variously treated IBDV on CSA cells. Virus growth of NCS and heated NCS treated IBDV was detected on both day-old and 3-week-old CSA cells. On the other hand, with the MN-Ab treatment, virus growth could only be observed in 3-week-old CSA cells, but was not detectable in day-old CSA cells, but the NCS treated IBDV was able to infect both day-old and 3-week-old CSA cells which were treated with heat-aggregated NCS. The CSA cells prepared from 1-week-old or older chickens were demonstrated to have rosette forming ability with AECS (Table 2). The phagocytic activity was observed in all CSA cells from chickens of different age groups using zymosan. These results indicate that the CSA cells possess phagocytic activity via the CR (Fig. 1) as early as one day old and the rosette forming ability of FcR can be detected in 1-week-old chickens.

Antibody response to IBD vaccination: The antibody responses of chickens to IBD vaccine are shown in Fig. 2. In SPF chickens, A, B and C groups, antibody response was observed at 7 days after vaccination. This antibody response was considered positive (over 0.2 in s/p ratio) and detected until 28 days after vaccination in both A and B groups. However, the C group chickens which were inoculated with MN-Ab treated live IBD vaccine, had lower antibody levels than both A and B groups. An s/p ratio of about 2.2 was obtained by ELISA. The mean s/p ratio of antibody response decreased with age in all commercial layer groups, but the antibody decay in subcutaneously vaccinated birds (E group) was statistically slower than in the birds in the unvaccinated (G, H) and orally vaccinated (F) groups, as shown in Fig. 2.

The challenge induced an antibody response in groups C, F and G, but no significant antibody response was observed in the subcutaneously vaccinated groups (A, B

Table 2. Rosette formation and phagocytosis of CSA cells prepared from SPF chickens of different ages

Age of chicken for CSA cells	No. of positive chickens with	
	Rosette formation ^{a)}	Phagocytic activity ^{b)}
Day old	0/5 ^{c)}	5/5
1 week old	4/5	5/5
2 weeks old	5/5	5/5
3 weeks old	5/5	5/5

a) Anti-sheep erythrocyte chicken serum was prepared to ascertain Fc receptor on the CSA cells.

b) Phagocytic activity with NCS treated zymosan to ascertain complement receptor on the CSA cells.

c) Five tested in each group.

Fig. 1. The phagocytic activity of NCS treated zymosan on CSA cells of day-old chickens demonstrated by fluorescent microscopy. $\times 600$.

and E).

Influence of vaccination on the complement activities: As shown in Fig. 2, neither CCP nor ACP activities were significantly altered after subcutaneous application of vaccine in day-old chickens. After challenge, the non subcutaneously vaccinated chickens had a decreased level.

Presence of IBDV and IBDV antigen in vaccinated chickens: As shown in Table 3, in each subcutaneously vaccinated A and E groups with untreated K strain, B group with NCS treated K strain and C group with MN-Ab treated K strain, IBDV was confirmed present in

Fig. 2. The kinetics of antibody response and complement activity by vaccinated and challenged chickens. SPF chickens were divided into four groups. A: Subcutaneous vaccination and challenge. B: Subcutaneous vaccination with NCS treated live vaccine and challenge. C: Subcutaneous vaccination with MN-Ab treated live vaccine and challenge. D: No vaccination and no challenge. Commercial layers were divided into four groups. E: Subcutaneous vaccination and challenge. F: Oral vaccination and challenge. G: Challenge only. H: No vaccination and no challenge. ● : S/p means $\pm$ SD ratio by commercial ELISA system. ○ : Hemolytic activity mean $\pm$ SD (CH_{50}) of classical complement pathway. —●— : Hemolytic activity mean $\pm$ SD (CH_{50}) of alternative complement pathway. ↓ : Challenge by virulent IBDV. Significantly (* $P < 0.01$) different from mean s/p ratio and hemolytic activity of complement in non-challenged group after challenge.

Table 3. Virus detection after vaccination and serological and pathological changes after challenge

Group ^{a)}	Maternal antibody	Virus detection after vaccination ^{b)}			2 weeks after challenge		
		Virus isolation	Immunofluorescent technique		FW/BW ^{c)} ratio	Antibody response ^{d)} by virulent IBDV	Pathological changes
			Bursa	Peripheral lymphocyte			
A	—	100%	60%	100%	0.53±0.05	Neg	—
B	—	100%	60%	80%	0.55±0.08	Neg	—
C	+	20%	20%	10%	0.16±0.07 ^{*e)}	2.64	+ ^{f)}
D	—	0%	0%	0%	0.51±0.12	Neg	—
E	+	100%	60%	100%	0.54±0.17	Neg	—
F	+	0%	0%	0%	0.25±0.08*	1.78	+
G	+	0%	0%	0%	0.27±0.11*	1.39	+
H	+	0%	0%	0%	0.45±0.07	Neg	—

a) One group: 5 chickens.

b) Smears of bursa and peripheral lymphocytes were collected at 14 days old.

c) (Bursa of Fabricius/body weight) ×100.

d) Antibody response is indicated by standard/positive ratio with ELISA. ELISA results are indicated according to the recommendation of IDEXX, Co., Ltd. Neg: <0.2 in s/p ratio.

e) Statistically significant ($P<0.01$) from bursa of Fabricius per body weight ratio for H group.

f) Histopathologically: intrafollicular cystic structure.

the bursae and in peripheral lymphocytes of 14 days old chickens. Virus antigen in the bursae of Fabricius was detected at 60% in A, B and E groups by FAT. On the other hand, the viral antigen in peripheral lymphocytes was detected at 80% or more in A, B and E groups (Fig. 3), but in subcutaneously vaccinated SPF chickens with MN-Ab treated IBDV only 20% or 10% viral antigen detection was achieved.

The effect of vaccination on clinical signs and pathology: None of the chickens in the eight experimental groups showed any clinical signs during the experimental period of 5 weeks.

However, the chickens in C, F and G groups had bursa/body weight ratios about 50% less than those of the other groups (Table 3). Histopathological changes typical of a wild type infection were observed in C, F and G groups. The bursae of Fabricius in C, F and G groups showed some intrafollicular cystic structures. In contrast, we failed to detect any histopathological changes in chickens in groups A, B and E.

DISCUSSION

Some reports indicated that canine distemper virus and dengue virus infected via FcR [1, 6], and epstein-barr virus and herpes simplex virus infected via CR [18, 20]. IBDV attacks IgM bearing B cells [9] and monocyte cells [4, 11]. Previously, we reported the possibility that IBDV neutralized by MN-Ab or/and complement could infect via FcR or/and CR in CSA cells of 3-week-old chickens [12, 13].

In this *in vitro* study, the infectivity of neutralized IBDV by NCS (as complement) to CSA cells was confirmed in day-old chickens. In the case of IBDV neutralized by MN-Ab, the infection was confirmed in chickens 1 week old or older. On the other hand, CSA cells of day-old chickens contain CR, but FcR was detected in 1-week-old chickens. The results of this *in vitro* study therefore

Fig. 3. Examination for viral antigen in vaccinated chickens by direct immunofluorescent antibody test. Smears of peripheral lymphocytes of subcutaneously vaccinated 14-day-old chickens. × 600.

suggested that the infection of neutralized IBDV by MN-Ab or/and NCS (as complement) on CSA cells is caused by CR and/or FcR and is an age dependent phenomenon.

In on *in vivo* study, SPF chickens which were subcutaneously inoculated with NCS neutralized IBDV, were positive for virus isolation and virus antigen at 14 days of age. Moreover, antibody levels of B group which was inoculated with NCS treated live IBD vaccine were detectable at 28 days of age. These results suggested that IBD vaccine virus had infected via CR on peripheral lymphocytes as the *in vitro* study. On the other hand, antibody response and pathological changes of in the bursae of Fabricius after challenge with virulent IBDV were not confirmed in SPF chickens subcutaneously inoculated with non- and NCS-treated live IBD vaccine. This indicated that live IBD vaccine neutralized by NCS was able to infect by subcutaneous application, and could

protect against the challenge by virulent IBDV of GBF-1. In commercial layers, subcutaneously vaccinated chickens had longer and higher antibody response than orally vaccinated and non vaccinated chicken groups at 25 days of age. Moreover subcutaneously vaccinated chickens were confirmed for virus isolation and virus antigen at 14 days of age. It was particularly interesting to note that chickens in E group had no secondary antibody response after challenge and no manifestation of severe type of infection with IBDV. These results demonstrate that subcutaneous application of the live IBD vaccine used in this experiment in the presence of MN-Ab can induce immunity to virulent IBDV infection.

Many reports had shown that mild type live IBD vaccine was not able to infect in the presence of MN-Ab [14, 16]. The data in this study showed that the vaccine strain of IBDV was able to infect immune cells via the CR or/and FcR and replicate. Moreover, the results of the *in vivo* study indicated that subcutaneous vaccination of day-old chickens in the presence of MN-Ab is able to protect against wild IBDV challenge at 21 days of age.

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REFERENCES

- Appel, M. J. B., Mendelson, S. G., and Hall, W. W. 1984. Macrophage Fc receptors control infectivity and neutralization of canine distemper virus-antibody complex. *J. Virol.* 51: 643-649.
- Box, P. 1989. High maternal antibody help chicks beat virulent virus. *World Poult.* 53: 17-19.
- Brown, F. 1984. Classification and nomenclature of viruses: Summary of results of meetings of the International Committee on Taxonomy of Virus in Sendai. September. *Int. Virol.* 25: 141-143.
- Burkhardt, E. and Muller, H. 1987. Susceptibility of chicken blood lymphoblasts and monocytes to infectious bursal disease virus (IBDV). *Arch. Virol.* 94: 297-303.
- Chettle, N. J., Stuart, J. C., and Wyeth, P. J. 1989. Outbreak of virulent infectious bursal disease in East Anglia. *Vet. Rec.* 125: 271-272.
- Halstead, S. B. and O'Rourke, E. J. 1977. Antibody-enhanced dengue virus infection in primate leukocytes. *Nature (Lond.)* 265: 739-741.
- Hirai, K., Shimakura, S., Chang, C. N., Adachi, Y., Kawamoto, E., Taguchi, N., Suzuki, Y., Itakura, C., Funakoshi, F., Tsushio, Y., and Hirose, M. 1973. Isolation of infectious bursal disease virus and distribution of precipitating antibodies in chicken sera. *Jpn. J. Vet. Sci.* 35: 105-113.
- Hirai, K., Shimakura, S., Kawamoto, E., Taguchi, N., Kim, S. T., Chang, C. N., and Iritani, Y. 1974. The immuno-depressive effect of infectious bursal disease virus in chickens. *Avian Dis.* 18: 50-57.
- Hirai, K. and Calnek, B. W. 1979. *In vitro* replication of infectious bursal disease virus established by lymphoid cell lines and chicken B lymphocytes. *Infect. Immun.* 25: 964-970.
- Hirai, K., Kunihiro, K., and Shimakura, S. 1979. Characterization of immunosuppression in chickens by infectious bursal disease virus. *Avian Dis.* 23: 950-965.
- Inoue, M., Yamamoto, H., Matsuo, K., and Hihara, H. 1992. Susceptibility of chicken monocytic cell lines to infectious bursal disease virus. *J. Vet. Med. Sci.* 54: 575-577.
- Komine, K., Ohta, H., Kamata, S., Uchida, K., and Hirai, K. 1989. Infectivity of infectious bursal disease virus neutralized by maternal antibody in various chicken cells. *Jpn. J. Vet. Sci.* 51: 634-635.
- Komine, K., Ohta, H., Kamata, S., Uchida, K., Yoshikawa, Y., Yamanouchi, K., and Okada, H. 1989. Complement activation by infectious bursal disease virus and its relevance to virus growth in the lymphoid cells. *Jpn. J. Vet. Sci.* 51: 1259-1262.
- Lukert, P. D. and Saif, Y. M. 1991. Infectious bursal disease. pp. 648-663. In: Disease of Poultry, 9th ed. (Calnek, B. W., Barnes, H. J., Beard, C. W., Reid, W. M., and Yorder, H. W. Jr. eds.), Ames, Iowa State University Press. U.S.A.
- Marquardt, W. W., Johnson, R. B., Odenwald, F. W., and Schlotthober, B. A. 1980. An indirect enzyme-linked immunosorbent assay (ELISA) for measuring antibodies in chickens infected with infectious bursal disease virus. *Avian Dis.* 24: 375-385.
- Naqi, S. A., Marquez, B., and Sahin, N. 1983. Maternal antibody and its effect on infectious bursal disease immunization. *Avian Dis.* 27: 623-631.
- Ohta, H., Yoshikawa, Y., Yamanouchi, K., and Okada, H. 1984. Lysis of horse red blood cells mediated by antibody-independent activation of the alternative pathway of complement. *Immunology* 49: 29-35.
- Nemerow, G. R., Wolfert, R., McNaughton, M. E., and Cooper, N. R. 1985. Identification and characterization of the Epstein-Barr virus receptor on human B lymphocytes and its relationship to the C3d complement receptor (CR2). *J. Virol.* 55: 347-357.
- Rabellino, E., Colon, S., Grey, H. M., and Unaue, E. R. 1971. Immunoglobulin on the surface of lymphocytes. I. Distribution and quantitation. *J. Exp. Med.* 133: 156-167.
- Smiley, M. L., Hoxie, J. A., and Friedman, H. M. 1985. Herpes simplex virus type 1 infection of endothelial and fibroblast cells induces a receptor for C3d. *J. Immunol.* 134: 2673-2678.
- Snyder, D. B., Marquardt, W. W., Mallinson, E. T., Savage, P. K., and Allen, D. C. 1984. Rapid serological profiling by enzyme-linked immunosorbent assay. III. Simultaneous measurements of antibody titers to infectious bronchitis, infectious bursal disease, and Newcastle disease viruses in a single serum dilution. *Avian Dis.* 28: 12-24.
- Takase, K., Nonaka, F., Fukuda, T., and Yamada, S. 1982. Recovery of virus from feces and tissues of chickens infected with cell-culture-adapted infectious bursal disease virus. *Jpn. J. Vet. Sci.* 44: 207-211.
- Tsukamoto, K., Tanimura, N., Hihara, H., Shirai, J., Imai, K., Nakamura, K., and Maeda, M. 1992. Isolation of

- virulent infectious bursal disease virus from field out breaks with high mortality in Japan. *J. Vet. Med. Sci.* 54: 153–155.
24. Van den Berg, T. P., Gonze, M., and Meulemans, G. 1991. Acute infectious bursal disease in poultry: isolation and characterization of a highly virulent strain. *Avian Pathol.* 20: 133–143.
25. Van den Berg, T. P. and Meulemans, G. 1991. Acute infectious bursal disease in poultry: protection afforded by maternally derived antibodies and interference with live vaccination. *Avian Pathol.* 20: 409–421.