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Effect of Bursectomy and Depletion of Immunoglobulin A on Antibody Production and Resistance to Respiratory Challenge After Local or Systemic Vaccination of Chickens with Newcastle Disease Virus

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Surgical removal of the bursa of Fabricius from newly hatched chicks resulted in a depletion of immunoglobulin A (IgA) from serum and bile of 55 and 67% of the birds, respectively, up to 11 weeks of age. The occurrence of IgG and IgM in serum and IgG in bile was not affected by neonatal bursectomy (Bx). A slight compensatory increase in the occurrence of IgM in bile was noted in Bx birds. When exposed to a lentogenic strain of Newcastle disease virus by either intramuscular or intratracheal routes, the Bx group produced a level of serum hemagglutinating antibody and tracheal wash neutralizing antibody indistinguishable from that reached in the sham-Bx group. All of the vaccinated Bx birds challenged by intratracheal exposure to a velogenic strain of Newcastle disease virus remained healthy. These results suggest that IgA is not essential for the development of immunity in the chicken and that other locally produced immunoglobulins or transuded serum antibody may protect the tracheal mucosa in the absence of IgA.

In humans levels of locally synthesized antibody have been correlated with resistance to viral infections of the respiratory and alimentary tracts (20, 29). Antibody of the immunoglobulin A (IgA) class has been found to be the predominant immunoglobulin in secretions of mucosal surfaces and, consequently, has been implicated as the mediator of immunity (19, 28). IgA was recently discovered to be among the chicken's repertoire of immunoglobulins present in serum, saliva, and bile as well as intestinal and oviduct secretions (2, 13, 15). However, the role of IgA in protecting the mucosal surfaces of the chicken from infections has not been established.

Since the discovery of the bursa of Fabricius as the source of stem cells committed to become immunoglobulin-producing plasma cells (5), numerous studies have used bursectomized (Bx) and X-irradiated birds to analyze the importance of antibody as a mediator of resistance to infection (4, 13, 23-25; G. Justines, Diss. Abstr., p. 419, 1971). Both Bx and X-irradiation of 1-day-old birds are required to eliminate both the source of bursa-derived cells and the bursa-derived cells that had already seeded to peripheral sites. A few reports have shown that Bx alone (11, 18) or Bx coupled with thymectomy (22) at 1 day of age is adequate to depress or prevent IgA synthesis without appreciably affecting synthesis of other classes of immunoglobulins. This effect was transient in some cases (18) and prolonged in others (11, 22).

The purpose of this study was to determine the effect that Bx followed by IgA depletion has on the antibody response of chickens vaccinated locally and systemically with Newcastle disease virus (NDV) and on the ability of these vaccinated birds to withstand challenge of the respiratory tract with a virulent strain of NDV. NDV was selected because of its predilection for infecting the mucosal surfaces of the respiratory tract of the chicken (10, 12). Our findings suggest that IgA plays, at best, a minor role in the overall protection of immunized birds from infection of the respiratory tract.

MATERIALS AND METHODS

Animals. Fertile Leghorn eggs were obtained from two sources: a commercial line, Shaver Starcross 288, from Perron Farms and Hatcheries Inc., Gainesville, Ga., and a pathogen-free line from Specific Pathogen Free Avian Supply (SPAFAS), Norwich, Conn. Both groups of eggs were set on the same day and hatched in isolation. Within 18 h after hatching, one-half of the birds from each source were surgically bursectomized, and the remaining one-half were sham-bursectomized. Bx was performed under Combuthal anesthe-

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sia (Diamond Laboratories, Des Moines, Iowa). Surgery was considered complete only if all of the bursa, including the stem, and a portion of the outer wall of the cloaca were removed. Birds surviving surgery were maintained in batteries in heated rooms supplied with filtered air and under positive pressure.

Vaccination and challenge. At 3 weeks of age 24 Bx and 24 sham-Bx birds from each source were divided into three groups. One group of 16 birds, 8 Bx and 8 sham-Bx, was injected intramuscularly (i.m.) with 0.2 ml of a B-1 strain of NDV (Sterwin Laboratories, Inc., Millsboro, Del.) having a titer of $10^{8.2}$ mean effective lethal doses per ml. A second similar group was given 0.4 ml intratracheally (i.t.) and 0.2 ml intranasally of the same stock of virus diluted 1:10 in sterile phosphate-buffered saline. The remaining birds, 8 Bx and 8 sham-Bx, served as uninoculated controls. Each vaccination group was housed in a separate modified Horsfall-Bauer unit supplied with filtered air and under positive pressure. At 6 weeks of age the two vaccinated groups were given a second dose of NDV. A La Sota strain of NDV (Sterwin Laboratories) containing $10^{8.4}$ mean effective lethal doses per ml was administered as previously described for each group.

Two weeks after the final vaccination a serum sample was obtained from each bird. Then four birds from each chicken line, both Bx and sham-Bx and representing each vaccination group (i.e., i.t., i.m., and controls), were challenged with a velogenic strain of NDV, Texas GB, which was administered i.t. and i.m. at a dosage approximately 1/100 of the optimal i.m. challenge dosage. The challenged birds were maintained in batteries housed in heated rooms supplied with filtered air and under negative pressure. Birds were bled and killed 3 weeks after challenge. Nonchallenged birds were killed, and serum, bile, and tracheal washes were obtained.

Collection of samples. Live birds were bled from the wing vein. Birds from which tracheal samples were taken were bled directly from the heart immediately after being killed. The trachea was exposed by making an incision along the ventral side of the neck with blunt-end scissors; clamps were placed at both ends, and the trachea was removed by transecting it just outside each clamp. The outside of the trachea was blotted with cotton gauze; then 4 ml of cold phosphate-buffered saline was slowly flushed through the lumen of the trachea into a glass tube. Samples were held on ice and subsequently lyophilized. The lyophilized samples were reconstituted with 1.0 ml of sterile distilled water before analysis.

Bile was aspirated from gallbladders with a syringe and needle, transferred to 1-dram (1.7-g) vials, and frozen.

Analysis of serum, bile, and tracheal wash fluids. Serum samples were analyzed for anti-NDV antibody by the hemagglutination inhibition test as described elsewhere (19), using 10 U of antigen per well. The plaque neutralization test was performed according to a standard procedure (6). Three-day-old monolayers of embryonic chicken kidney cells were inoculated with twofold dilutions of tracheal wash samples preincubated with a standard dilution of indicator virus (Kansas-Manhattan strain of NDV). Plaques were visualized after 72 h of incubation by the addition of neutral red, and the mean number of plaques on duplicate plates for each dilution was determined. The titer was determined as the highest dilution of sample that yielded between 45 and 55% as many plaques as found on plates inoculated with a mixture of indicator virus and negative serum. Selected tracheal wash samples were assayed for interferon activity by the inhibition of plaque formation by vesicular stomatitis virus as described elsewhere (8).

Immunoglobulins in serum and bile were detected by double diffusion in gel according to the method of Ouchterlony (21), using class-specific antisera. Gels contained 1% agarose, 0.8% NaCl, and 2% polyethylene glycol 6000 dissolved in 0.01 M phosphate buffer, pH 7.5. Under optimal conditions this test was capable of detecting as little as 50, 30, and 60 μg of IgG, IgA, and IgM, respectively, per ml. Because of our inability to obtain a pure preparation of IgM, this value is, most likely, an underestimate of the sensitivity for this immunoglobulin.

Preparation of antisera. Serum from 1-day-old chicks, which contained IgG but no IgM or IgA, was the source of IgG. Serum from adult birds was used to obtain IgM. IgA was isolated from the bile of 8-week-old chickens. The globulin fractions were precipitated from sera after the method of Benedict (1), and purified IgG and partially purified IgM were obtained by repeated gel filtration with Sephadex G-200 columns. The fraction of adult serum that eluted with the void volume of the G-200 column contained all three classes of immunoglobulin. Separation of IgG from IgM was achieved by passage over an agarose A-1.5 column; however, the IgM-enriched preparation, which eluted in the leading peak, still contained some IgA. Bile globulins were separated from low-molecular-weight bile salts essentially according to the methods of Lebacq-Verheyden et al. (14) by making a 50% dilution of the bile with 0.015 M tris(hydroxymethyl)aminomethane-0.06 M NaCl (pH 7.4) buffer and passage over a Sephadex G-75 column. The leading peak of protein was then passed over an agarose A-1.5 column. The leading peak was shown to contain IgA and no IgG or IgM when analyzed by immunoelectrophoresis with rabbit anti-whole chicken serum or by double diffusion in gel using class-specific antisera.

The preparations of IgG, IgM, and IgA were mixed with complete Freund adjuvant and injected into multiple intramuscular sites of individual goats. Subsequent injections were given at 4-week intervals with incomplete Freund adjuvant. The same preparations of IgG, IgM, and IgA used for immunization, as well as agammaglobulinemic plasma (kindly supplied by D. Y. E. Perey) and serum from 1-day-old chickens, were coupled to Sepharose 4B after cyanogen bromide activation (17) and used as solid immunoabsorbents. The goat antiserum to IgG was absorbed consecutively with the agammaglobulinemic plasma, IgM, and IgG immunoabsorbents. After extensive washing with tris(hydroxymethyl)aminomethane-NaCl buffer, pH 7.2, antibodies bound to the IgG immunoabsorbent were eluted with 3 M NaSCN dissolved in the same buffer. After elution the antibodies were dialyzed extensively against tris(hydroxymethyl)aminomethane-

NaCl to remove thiocyanate ions. The same technique, with the following sequence of immunoabsorbents, agammaglobulinemic plasma, 1-day-old-chicken serum, IgA, and IgM, was used to purify antibody to μ chains; and agammaglobulinemic plasma, 1-day-old-chicken serum, and IgA were used for isolation of antibodies to α chains. The specificity of the resultant antisera for isotype determinants was demonstrated by double diffusion in gel and immunoelectrophoresis. Also, antibody preparations were coupled with fluorescein isothiocyanate and with tetramethylrhodamine isothiocyanate as described by Brandtzaeg (3). Each antibody preparation labeled with contrasting flurochromes was shown to stain different plasma cells in sections obtained from the gland of Harder, without overlapping of stains.

RESULTS

The effect of surgical Bx of 1-day-old chickens on subsequent immunoglobulin production is given in Table 1. Except for one Bx bird in which no IgM could be detected, Bx did not alter the appearance of IgG or IgM in the serum of chickens. Although IgA was consistently detected in the serum of sham-Bx birds, only 45% of the Bx birds had detectable levels of IgA in serum. In bile, IgG and IgM were only occasionally detected in sham-Bx birds. Bx did not appreciably alter the frequency of IgG detection in bile; however, the frequency of IgM detection was increased 27% in Bx birds. On the other hand, IgA, which was consistently detected in the bile of sham-Bx birds, was only found in 33% of the bile samples of Bx birds. Since no difference in the effect of Bx on immunoglobulin production was found between the two bird lines used in this study, the results from each group were pooled to give the ratio found in Table 1.

The results of vaccination and subsequent challenge of Bx and sham-Bx birds are given in Table 2. The plaque neutralization titers of tracheal washes and serum hemagglutination inhibition titers are given as the geometric mean of four samples collected from each group. Low levels of neutralizing activity were detected in the tracheal washes of both i.m.- and i.t.-vaccinated birds. Among the i.m.-vaccinated group the titers were essentially the same for Bx or sham-Bx birds. However, the mean titers were almost two dilutions higher for the Bx i.t.-vaccinated birds when compared with the sham-Bx birds of the same group.

Too few birds of the commercial (Shaver) line survived surgery to be included in a nonchallenged sham-Bx group. Therefore, although a moderate level of plaque-neutralizing activity was detected in the tracheal washes of Bx commercial birds, no comparison could be made

TABLE 1. *Immunoglobulins detected in the serum and bile of Bx and sham-Bx chickens at 8 weeks of age*

Sample	Immunoglobulin tested[a]	Immunoglobulin detected/total tested		Change (%)[b]
		Sham-Bx	Bx	
Serum	IgG	46/46	55/55	0
	IgM	46/46	54/55	− 0.8
	IgA	46/46	25/55	−55
Bile	IgG	6/21	8/27	+ 1
	IgM	3/21	10/27	+27
	IgA	21/21	9/21	−66

[a] Assay sensitive to 50, 60, and 30 μg of IgG, IgM, and IgA, respectively, per ml.

[b] Change in the percentage of positive samples obtained for the Bx group relative to the sham-Bx group.

TABLE 2. *Immune response of Bx and sham-Bx chickens vaccinated with NDV vaccine.*

Chicken line	Vaccination route	Bx	Prechallenge		Postchallenge	
			Tracheal wash plaque neutralization titer[a]	Serum HI titer[a]	Survivors (%)	Serum HI titer[a]
SPAFAS	i.m.	Bx	2.4	67	100	67
	i.m.	Sham-Bx	2.7	80	100	160
	i.t.	Bx	16.0	44	100	135
	i.t.	Sham-Bx	5.3	92	100	95
	Control	Bx	2.0	5	0	NT[b]
	Control	Sham-Bx	2.0	5	0	NT
Shaver	i.m.	Bx	9.5	54	100	95
	i.m.	Sham-Bx	NT	160	100	121
	i.t.	Bx	6.0	66	100	89
	i.t.	Sham-Bx	NT	101	100	127
	Control	Bx	2.0	5	0	NT
	Control	Sham-Bx	2.0	5	0	NT

[a] Reciprocal of geometric mean titer. HI, Hemagglutination inhibition.

[b] NT, Not tested.

with a sham-Bx group from the same chicken line.

The serum antibody response varied between the two lines of birds. Among the SPAFAS birds very little difference was found between the mean titers of either i.m.- or i.t.-vaccinated birds before challenge. However, the mean titer of the sham-Bx birds was slightly higher than that of the Bx birds. All the vaccinated birds, both Bx and sham-Bx, survived challenge with no symptoms of infection. The mean titers of the Bx i.m.-vaccinated birds and the sham-Bx i.t.-vaccinated birds remained unchanged after challenge. On the other hand, the sham-Bx i.m.-vaccinated birds and the Bx i.t.-vaccinated birds showed a rise in serum hemagglutination inhibition antibody after challenge.

Among the commercial Leghorns the serum hemagglutination inhibition titers of the Bx birds were consistently lower than those of the sham-Bx birds for both the i.t.- and the i.m.-vaccinated birds before challenge. Also, as in the SPAFAS line, both routes of vaccination produced similar antibody titers, and all vaccinated birds survived challenge.

DISCUSSION

Bx at 1 day of age resulted in a selective depletion of IgA from serum of at least 55% of the birds treated. These findings are generally consistent with the results of other workers who found a selective depression of IgA synthesis in neonatally Bx chicks (11, 16). Unlike the observation of Perey and Bienenstock (22), thymectomy in addition to Bx was not required to prevent IgA synthesis.

Also, IgA in bile, a secretion normally having extremely high levels of IgA (15), was undetectable in 67% of the Bx birds. This finding is consistent with the observed elimination of IgA from saliva in 50% of the birds bursectomized by Leslie and Martin (16). However, the slight increase in the occurrence of IgM that we observed in bile of Bx birds was not reported to have been the case in saliva (16). In humans deficient in IgA, IgM has been found to commonly replace IgA in secretions (7, 27). Furthermore, the failure to detect IgA in serum and bile of Bx birds has recently been correlated with an absence of IgA plasma cells in the glands of Harder, cecal tonsils, and spleen (unpublished observations). Similar observations were reported previously for the cecal tonsils (11).

Bx only slightly depressed antibody production in both i.m.- and i.t.-vaccinated birds. This finding is in contrast to the severe depression or ablation of antibody production caused by either hormonal Bx (25; Justines, Diss. Abstr., p. 419, 1971) or combined Bx and X-irradiation (4, 24). In the present study, peripheral lymphoid organs that had been seeded with bursa-derived cells before hatching were left intact. Therefore, the humoral immune system was not severely restricted.

The serum antibody levels of the commercial Leghorns were essentially the same after challenge, indicating that sufficient antibody was present to block stimulation of the immune system. Similar results were observed with the SPAFAS birds, except that birds possessing relatively low levels of antibody before i.t. challenge did show a rise in serum antibody levels, probably due to insufficient neutralization of the challenge virus.

By selectively restricting or eliminating the IgA-synthesizing capacity of chickens it was expected that the local immune mechanisms that in other species are primarily mediated by IgA would be depressed. The only study similar to this one was reported by Perey and Dent (24). They found that birds rendered dysgammaglobulinemic by Bx at 1 day of age and subsequently vaccinated were just as susceptible to the lethal effects of i.m. challenge with a virulent strain of NDV as agammaglobulinemic birds. Since the challenge virus was given by the i.m. route, no conclusions could be drawn concerning the effect of Bx on local immune mechanisms.

In the study reported herein no significant effect of Bx and the resulting dysgammaglobulinemia could be detected either on the appearance of neutralizing antibody in tracheal washes or on the resistance of vaccinated chickens to i.t. challenge with a virulent strain of NDV. Several possible explanations may be given for these findings: (i) antibody of classes other than IgA may have been synthesized locally and may have mediated local resistance; (ii) cell-mediated immune mechanisms may have played a major role in the defense of mucosal surfaces; (iii) the test used to determine resistance, i.e., death as a result of challenge, may not have been sensitive enough to detect a subtle effect of IgA depletion on the immune system; (iv) serum antibody may have masked the effect of IgA depletion by neutralizing the virus both in the trachea, by transudation of antibody from serum, and in circulation.

In general, it appears that the depression of IgA synthesis does not prevent the development of immunity to NDV administered by either i.m. or i.t. inoculation. These findings are in accord with the observation that humans who either lack IgA or have IgA deficiency have no problem recovering from upper respiratory tract infections (9, 26).

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