Vaccination of Congenitally-Immune Chicks against Newcastle Disease Virus (33057)

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Young chicks with congenitally derived antibodies to Newcastle disease virus (NDV) are refractory to early vaccination (1–6). This refractoriness is generally attributed to incomplete immunological competence of the chicks and to an abrogating effect of maternal antibodies on the vaccine antigen. Active immunization of these chicks has been satisfactorily achieved only by intranasal inoculation with live (usually attenuated) virus. Lancaster (7) has recently presented a comprehensive review of the problems of establishing a uniformly successful vaccination procedure.

For broiler chicks, satisfactory immunity, i.e., >80% protection, is desired up to 8 or 10 weeks of age. Levels of congenital immunity in commercial hatches are rarely this high and, moreover, this protection is short-lived. Recently, interest has been revived in the prophylactic utility of both convalescent NDV-immune serum and γ-globulins from fractionated antisera (8). Passive immunity conferred by these preparations is frequently incomplete, particularly when challenged with highly virulent (velogenic) strains of NDV.

We have tried to develop a simple vaccination procedure for broiler chicks combining both passive and active immunization in a single subcutaneous (s.c.) inoculum. Our objective was to achieve early, sustained prophylaxis that would provide >80% protection for 8–10 weeks. The vaccine should be noninfectious and useful for both susceptible chicks and those with maximal or nominal levels of congenital immunity.

We report here the achievement of protective levels of immunity with a vaccine containing chemically-inactivated virus antigen in the form of antigen–antibody complexes (Ag·Ab), free homologous antibody, and an aluminum hydroxide adjuvant. This admixture of components was chosen because it might be expected to fulfill two conditions considered paramount for a satisfactory combination vaccine: (i) the humoral level of congenitally acquired antibody should not be lowered as a result of vaccination, and (ii) the form and quantity of antigen administered should insure its persistence in the chick beyond any refractory period. A variety of control preparations evaluated the effectiveness of this combination vaccine.

Materials and Methods. Antigen and antibody. The GB-Texas strain of NDV (9) was used exclusively. It was propagated in 10-day embryonating eggs and attained titers of $10^{9.5}$. ELD_{50}/ml in the allantoic fluid (AF) at harvest (40- to 48-hour incubation, 0.1 ml of inoculum containing $10^{4.5}$ ELD_{50}). Pooled AF virus was inactivated with 0.2% (v/v) β-propiolactone (BPL for 2 hours at 25°C and stored at —60°C until used as antigen. Homologous antibody was obtained from chickens vaccinated subcutaneously (s.c.) with a standard killed NDV vaccine (11) and subsequently hyperimmunized with a challenge inoculation of $10^{4.5}$ BLD_{50} given intramuscularly (i.m.). Antisera were collected 2 weeks after this challenge. Antibody, as immune globulins, was separated from pooled antisera by ammonium sulfate precipitation (⅓ saturation, 0°C, 3 times precipitated). Globulin precipitates were reconstituted to the original serum volume with 0.85% NaCl (saline), and then dialyzed against saline for 4 days at 4°C. When assayed for hemagglutination inhibition (HI) by the constant virus-decreasing serum method (10), the globulins contained 1280 HI units/ml, i.e., about 50% of the HI activity of the original pooled antiserum.

Experimental chicks. White leghorns, 1–2 days old, were used. They represented chicks of four different immune levels and were tested with the experimental vaccine as separate groups. These groups were: susceptible

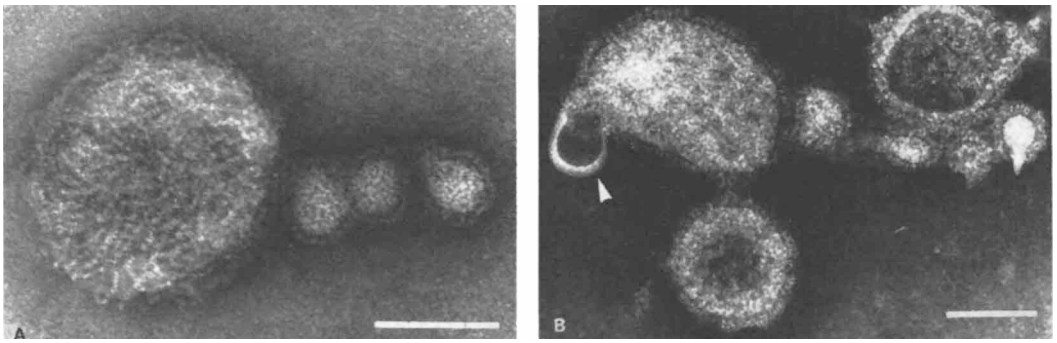

FIG. 1. A. Newcastle disease virus antigen in potassium phosphotungstate-negative stain. Intact antigen particle and three hemagglutinins ('rosettes'). B. Newcastle disease virus antigen–antibody complexes in potassium phosphotungstate-negative stain. Note that the blebbed portion (arrow) of the large antigen particle at the left is devoid of antibody. Scale bar represents 50 mμ.

chicks, chicks of nominal immunity, congenitally-immune chicks protected to an intermediate level against NDV, and high level congenitally-immune chicks. Susceptible chicks were obtained from a flock that had no history of Newcastle disease or NDV vaccination.

Chicks of nominal passive protection were produced artificially from susceptible chicks by s.c., injection of NDV antiserum in the sternum region (640 HI units, 0.5 ml). They were used in experiments 1 day later. Twenty percent of these chicks survived a challenge inoculation of $10^{4.5}$ BLD_{50} 2 weeks later.

Congenitally-immune chicks with an intermediate level of immunity were obtained from hens vaccinated twice with killed NDV vaccine (11). Fifty-two percent of these chicks were immune to challenge at 2 weeks of age. Six comparable hatches of chicks were used over a 13-month period.

Congenitally-immune chicks with high levels of protection were obtained from a flock that had been vaccinated with killed NDV vaccine (11) and subsequently hyperimmunized with a live virus challenge. Chicks in this group were 100% immune to challenge up to 3 weeks of age. Various hatches of these chicks, used over an 18-month period, had comparable levels of congenital immunity.

Experimental, antigen–antibody complex vaccine. Preliminary trials (unpublished) indicated that the following volume ratio of components was satisfactory: 50:5:45 (BPL-killed AF virus antigen:immune globulin preparation:adjuvant). To form the Ag·Ab complexes, antigen and the immune globulin preparation were mixed in the ratio 50:5 and incubated at room temperature for 2–3 hours and then overnight at 4°C. After incubation, this reaction mixture contained 40 *HI* units/ml indicating an antibody excess.

Electron micrographs of Ag·Ab complexes showed that antigen particles were covered with a thick, matted layer (presumably antibody) not present on antigen particles before incubation with immune globulins (Fig. 1).

The complete vaccine was compounded by blending 55 parts of the Ag·Ab complex preparation with 45 parts of the aluminum hydroxide adjuvant (Amphojel[1]) in a Waring Blendor[2] (0°C, 3–5 min, high speed setting). The vaccine was generally used immediately although some lots were used after storing for several months at 4°C. No loss in its immunogenic potency was noted during this storage.

Control preparation. Compositions of the control formulations were as follows: (i) 50 parts BPL-killed antigen:5 parts phosphate buffered saline (PBS):45 parts adjuvant; (ii) 50 parts BPL-killed antigen:5 parts *normal* chicken globulin:45 parts adjuvant; (iii) 50 parts BPL-killed antigen:5 parts immune globulins:45 parts PBS; (iv) 55 parts

[1] Wyeth Laboratories, Inc., Philadelphia, Pennsylvania.

[2] Waring Corporation, New York, New York.

PBS:45 parts adjuvant; (v) 5 parts immune globulins:95 parts PBS; and (vi) 5 parts immune globulins:45 parts adjuvant:50 parts PBS. In control Nos. 2 and 3, antigen and globulin preparations were incubated together as described for the test vaccine. All control formulations were compounded by blending as described for the test vaccine.

The complete spectrum of these controls was used only in experiments with susceptible chicks. For chicks with a nominal level of passive immunity, all formulations except No. 1 were used. For chicks with high levels of congenital immunity, only control formulation No. 1 was used, i.e., antigen:PBS:adjuvant but without immune globulins.

None of these special control preparations was tested in chicks with an intermediate level of congenital immunity. In all experiments, however, groups of nonvaccinated control chicks representing the appropriate level of immunity were challenged.

Vaccine administration and challenge. The test vaccine and control formulations were given in a 1.0-ml volume applied s.c. in the dorsal region of the neck. This route was chosen to provide a slow release of the immunogen into the lymph and blood since Brunner *et al.* (12) have shown that NDV–Ag·Ab complexes are cleared more rapidly from the blood (in mice) than native antigen. Furthermore, it was known that antibody synthesis may be suppressed by intravenous (i.v.) administration of Ag·Ab complexes formed in the region of antibody excess with other antigens (13–15).

Beginning at 2-weeks postvaccination and following at 1- to 4-week intervals thereafter, chicks in each immune level group were given an i.m. challenge injection of $10^{4.5}$ BLD_{50} of the GB-Texas strain. Those that survived 2 weeks and did not exhibit nervous signs or paralysis were considered protected.

Nonvaccinated susceptible chicks were also included in each challenge to confirm its effectiveness.

Results. All nonvaccinated *susceptible* chicks used as controls died from the challenge dose $10^{4.5}$ BLD_{50}, of the GB-Texas strain.

Challenge response of high level immune chicks. Chicks given the experimental Ag·Ab complex vaccine maintained greater than 75% protection throughout the 10-week test (Table 1-A and Fig. 2). Chicks given control formulation No. 1 containing antigen and adjuvant *but without antibody* did not maintain a comparable level of protection, e.g., at 4-weeks postvaccination (PV) only 50% survived challenge and, by the tenth week, less than 30% survived. The congenital immunity of nonvaccinated controls appeared to persist for about 3 weeks; however, it waned thereafter and less than 50% survived challenge at 6-weeks PV or later.

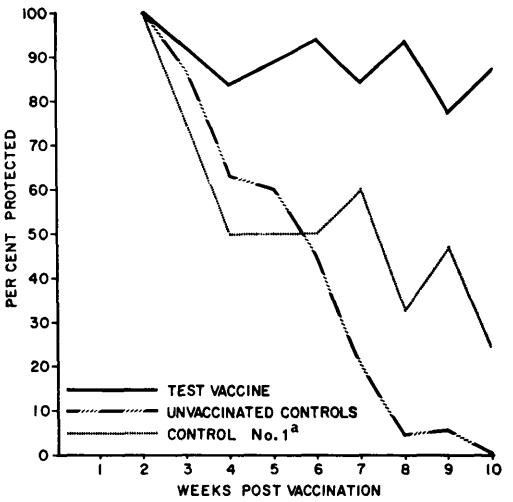

FIG. 2. Challenge response of 1- to 2-day-old high level congenitally immune chicks after vaccination with antigen–antibody complex vaccine.

[a]Composition of control No. 1, antigen:PBS:adjuvant (50:5:45).

Challenge response of intermediate level immune chicks. Chicks given the Ag·Ab complex vaccine maintained greater than 80% protection throughout the 10-week test except those challenged at 3-weeks PV (Table 1-B and Fig. 3). At this challenge, only 65% survived. Less than 50% of the nonvaccinated congenitally-immune controls survived challenge after 2 weeks and their protection was nil after 5 weeks.

Challenge response of nominally-immune chicks. These chicks responded to the Ag·Ab complex vaccine somewhat better than the high level congenitally-immune chicks, particularly from the fourth week PV; during the last 5 weeks of the test their protection was

TABLE I. Challenge Response of "Naturally Immune" Chicks after Vaccination Against Newcastle Disease Virus with an Experimental Antigen–Antibody (Ag • Ab) Vaccine.

Inoculum	Weeks:	Postvaccination (no. of survivors/no. challenged)								
		2	3	4	5	6	7	8	9	10
A. High level congenitally-immunes										
Complete Ag • Ab vaccine[a]		12/12		10/12		17/18	20/24	14/15	17/22	15/17
Antigen, PBS, and adjuvant[a]		10/10		6/12		9/18	15/25	5/15	8/17	4/16
Nonvaccinated controls[b]		35/35	13/15	5/8	30/51	26/61	16/76	4/76	2/30	0/14
B. Intermediate level congenitally-immunes										
Complete Ag • Ab vaccine[c]		18/20	36/55	58/70	29/34	15/16	17/21		36/42	14/17
Nonvaccinated controls[d]		12/23	17/38	9/54	2/40	0/31	0/17		0/26	0/25
C. Nominal level artificially-immunes										
Complete Ag • Ab vaccine		15/19	15/18	18/18	19/20				14/15	
Antigen, normal globulin, and adjuvant		7/20	4/18	6/17	14/19				14/15	
Antigen, immune globulin, and PBS			1/20							
Immune globulin and PBS only			2/20							
Immune globulin, adjuvant, and PBS			1/19							
Nonvaccinated controls		4/20	0/20	0/20	0/20				0/20	
D. Susceptibles										
Complete Ag • Ab vaccine		17/19	15/16	20/20	20/20				14/14	
Antigen, PBS, and adjuvant		20/20	19/20	15/16	20/20				19/20	
Antigen, normal globulin, and adjuvant		19/20	20/20	19/19	20/20				19/20	
Antigen, immune globulin, and PBS			0/20							
Adjuvant and PBS only			0/16							
Immune globulin and PBS only			0/20							
Immune globulin, adjuvant, and PBS			0/18							
Nonvaccinated controls		0/10	0/10	0/10	0/10				0/10	

[a] From 4 hatches.
[b] From 16 hatches.
[c] From 3 hatches.
[d] From 6 hatches.

greater than 90% (Table 1-C and Fig. 4). In contrast, the nonvaccinated artificially-immune controls all died from challenge after the second week PV. With one exception, described below, none of the control formulations elicited an immune response in these chickes. Chicks given control preparation No. 2 containing antigen, adjuvant but with "normal chicken globulins" as a substitute for homologous antibody, responded in an unexpected manner; during the first 4 weeks PV, less than 40% survived challenge whereas at 5 weeks PV or later, greater than 70% survived (Fig. 4).

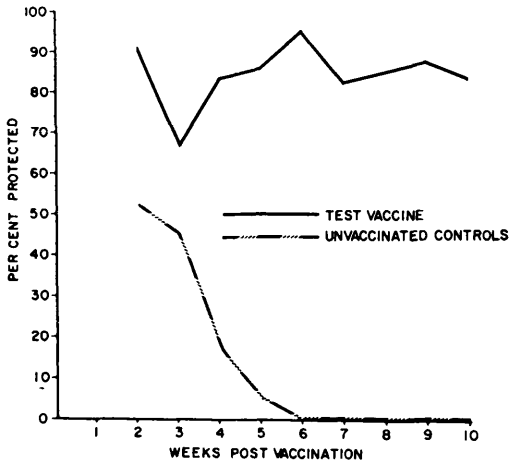

FIG. 3. Challenge response of 1- to 2-day-old intermediate level congenitally-immune chicks after vaccination with antigen–antibody complex vaccine.

Challenge response of susceptible chicks. The experimental Ag·Ab complex vaccine and Nos. 1 and 2 control formulations induced greater than 85% protection throughout the test (Table 1-D and Fig. 5). The two control formulations did not contain homologous antibody but appeared to afford a higher level of protection (95–100%) than the test vaccine up to the fourth week PV. All nonvaccinated chicks and those receiving control preparations that did not contain *both* antigen and adjuvant died as a result of the challenge at 3 weeks.

Discussion. Our observations suggest that the apparent immunological refractoriness of day-old NDV congenitally-immune chicks can be overcome by appropriate vaccination

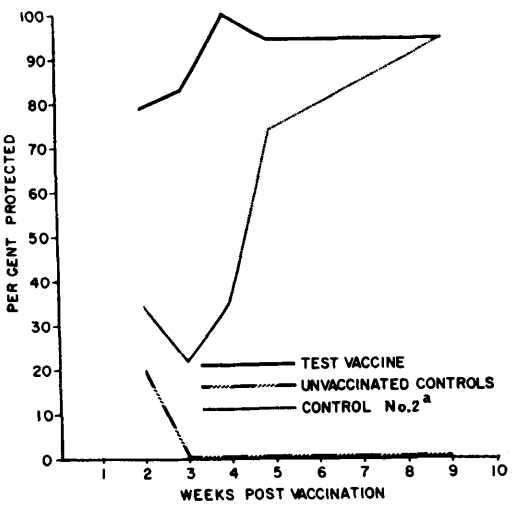

FIG. 4. Challenge response of 1- to 2-day-old nominal level artificially-immune chicks after vaccination with antigen–antibody complex vaccine.

[a] Composition of control No. 2, antigen:normal chicken globulins:adjuvant (50:5:45).

with chemically-inactivated virus antigen in the form of Ag·Ab complexes, excess homologous antibody, and an adjuvant. This combination of components was also immunogenic in susceptible chicks but afforded somewhat

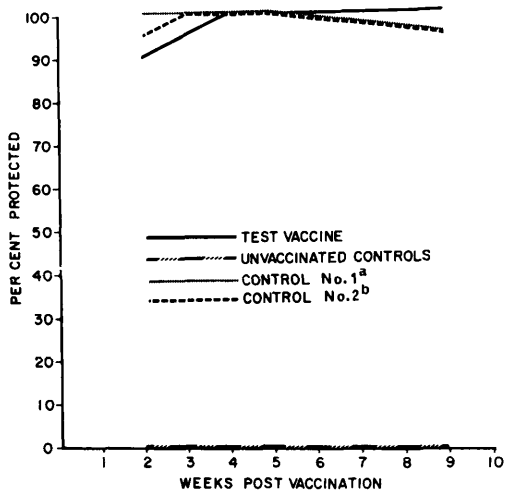

FIG. 5. Challenge response of 1- to 2-day-old susceptible chicks after vaccination with antigen–antibody complex vaccine.

[a] Composition of control No. 1, antigen:PBS:adjuvant (50:5:45)

[b] Composition of control No. 2, antigen:normal chicken globulins:adjuvant (50:5:45).

less protection than simple antigen–adjuvant vaccines up to 4-weeks PV. This result may be due to a temporary conference of refractoriness by the antibody given in the experimental vaccine.

The factors that may have contributed to overcoming the chick's refractoriness have not been clearly identified. It seems reasonable to us, however, that both passive immunity and antigen persistence were important vaccination parameters. Since the Ag·Ab complex vaccine contained unbound antibody, we assume the level of congenitally-derived antibody was not lowered as a result of vaccination. Passive immunity may have been enhanced by the unbound antibody. However, vaccine-equivalent concentrations of immune globulin in control preparations given to susceptible chicks and nominally-immune chicks did not confer protection beyond 3 weeks. It seems unlikely that this additional passive immunity could, *alone,* account for the sustained high level of protection afforded by the experimental vaccine. The effective concentration of immunogenic components of the experimental vaccine may have been increased by comminution of the Ag·Ab complex aggregates in the blendor. In addition to the well-known adjuvant effect of aluminum hydroxide gels, the presence of a thick, matted covering of antibody may have enhanced antigen persistence by at least three mechanisms: (i) stabilization of the antigen particles against disruption by normal chicken globulins, which induce pleomorphic changes and degradation (16); (ii) reduction of the rate of antigen diffusion from the injection site; and (iii) masking of the identification of the antigen as a "foreign substance" thereby reducing the rate of its clearance by phagocytosis. If these assumptions are correct, immunological refractoriness in other species and with other infectious entities may be overcome by a similar approach in vaccine development.

Summary. One to 2-day-old chicks with different levels of passive immunity and controls were vaccinated with an homogenate of chemically-inactivated Newcastle disease virus antigen–antibody complexes, excess antibody, and aluminum hydroxide adjuvant. The antigen–antibody complex vaccine protected 65% or more of the chicks (usually $> 80\%$) against a challenge dose of $10^{4.5}$ BLD_{50} for at least 10-weeks postvaccination. Comparable protection was not achieved in the passively-immune chicks when vaccinated with preparations containing only antigen and adjuvant. In susceptible chicks, however, the antigen–antibody complex vaccine did not confer any better protection against challenge inoculation than did simple vaccines. It was concluded that immunological refractoriness of congenitally-immune chicks can be surmounted by appropriate vaccination with an antigen–antibody complex vaccine.

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