PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 148, 365–369 (1975)

Host Resistance Mechanisms to Newcastle Disease Virus in Immunodeficient Chickens[1] (38540)

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(Introduced by John Bienenstock)

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Clinical experience with immunodeficiency syndromes strongly suggests that recovery from a wide variety of viral infections in man is closely linked to an intact capacity to elaborate thymus-derived T cell immunity (1, 2). Conversely, individuals with isolated bursa- or bone marrow-derived B cell deficiency successfully recover from viral infections but are markedly susceptible to extra cellular pathogens (3).

Experimental animal studies attempting to determine the respective role of T and B cells in host resistance mechanisms against viruses have led to conflicting results and interpretations possibly as a consequence of the different hosts, viruses and experimental protocols being compared. Because of the possibility of selectively manipulating the thymus-dependent and bursa-dependent immune systems in the chicken, this species is one of the best animal models to assess the relative significance of T and B cell immunity during host-virus interactions. Cheville and Beard (4) have used immunodeficient chickens and studied their capacity to resist exposure to an avian pathogen: Newcastle disease virus (NDV). These workers used neonatally bursectomized or thymectomized birds which were also sublethally X-irradiated. Several weeks later, both groups of birds successfully resisted infection with an avirulent or lentogenic Bl strain of NDV, however conflicting data were obtained regarding the possible protective effect of such immunization when the birds were subsequently challenged intranasally (in) or im with a virulent strain of the same virus. Moreover, the role of cell mediated immunity to NDV could not be assessed conclusively, since neonatal thymectomy was incomplete and all birds, regardless of earlier manipulations developed delayed hypersensitivity reactions to NDV. Using an enterotropic strain of NDV, Kono *et al.* (5) concluded that B cell deficient chickens were more susceptible to the disease than were T cell deficient and control birds.

We report, herein, initial studies of Newcastle disease in immunodeficient chickens inoculated in with mesogenic NDV. This virus only kills 10–15% of normal chickens after inoculation over a wide dose range. Our results strongly suggest that humoral immunity to NDV is a vital component of the host resistance mechanisms to this virus, however T cell immunity may also play a definite, though lesser role in resisting initial infection.

Materials and Methods. Fertile eggs from a cross of two inbred lines of White Leghorn chickens (line WC, Hy-Line International, Johnston, IA) were incubated under standard conditions. Newly hatched chicks were surgically bursectomized or thymectomized (6) under combutal anesthesia (Diamond Laboratories, Downsview, Ontario). Control animals were sham-operated under similar conditions and all birds were exposed to 750 R total body X-irradiation (7) 1 day later. The conditions of irradiation were: 250 kvp, 30 mA, 0.5 mm copper and 1.0 mm aluminum filters, 1.43 mm copper half value layer. Chicks were restrained and placed on a turn table rotating at 8 rpm and exposed under maximum back scatter condition to a vertical beam of X-rays delivered by a General Electric Maxitron 250 machine. The distance from the focal point to mid-plane was 80 cm; the tissue dose rate measured at mid-plane was 54.1 R/min.

Ten weeks after irradiation, agammaglobulinemic (Aγ) birds were identified by screening their sera in double gel diffusion

[1] Supported by the Medical Research Council of Canada.

against monospecific anti-μ and anti-γ chain antisera (8). T cell function was assessed in thymectomized–irradiated birds and their controls, by determining the response of cultured blood lymphocytes to concanavalin A (con A) stimulation since it has been shown to represent a selective T cell response (9). Fifty μl of whole heparinized blood were added to triplicate tubes containing 2 ml of serum-free culture medium (RPMI 1640) to which 0, 10 or 100 μg/ml con A were also added. Lymphoid cell proliferation as reflected by DNA synthesis was assessed by measuring incorporation of tritiated thymidine (2 μCi/ml, sp act 6.7 Ci/m*M*, NEN-Canada Ltd., Montreal, Quebec) during the final 4 hr of a 72 hr culture period.

Newcastle disease virus was grown and titrated in 10-day-old embryonated eggs. The virus, obtained from Dr. P. Plagemann, Department of Microbiology, University of Minnesota, was derived from a well characterized Bl stock. However, after several passages in mouse fibroblasts, as well as in embryonated eggs, this virus has acquired increased virulence as judged by a mean death time of embryos of 59.8 hr. Moreover, sporadic mortality of NDV-infected normal young adult chickens has been observed as well as cytopathogenicity *in vitro* on chick embryo fibroblasts. We have interpreted these findings as indicating that the virus now behaves as a mesogenic strain (10) and have designated it as NDV_{Plag}.

In addition, we have used the vaccine strain (Bl) of NDV which we purchased from Connaught Medical Laboratories, Toronto, Ontario. Unless otherwise indicated, all birds were inoculated intranasally with various dilutions of freshly thawed virus stocks before being placed in isolators. Plasma was collected from all birds before inoculation and at weekly intervals thereafter. Anti-NDV antibodies were titrated in a standard hemagglutination inhibition (HI) microassay.

In the second part of our studies, attempts were made to immunize normal and immunodeficient chickens using either the avirulent vaccine strain or β-propriolactone (BPL) inactivated NDV_{Plag} (11) emulsified in complete Freund's adjuvant (CFA) injected im.

Results. The responses of peripheral blood lymphocytes to con A stimulation could easily differentiate neonatally thymectomized–irradiated (Tx–X) birds (T cell deficient) from sham operated–irradiated control birds (STx–X). Indeed, Tx–X birds had a response which was considerably lower than that of the control birds (mean cpm $\pm$ SE = 4320 $\pm$ 2086 compared to 119,713 $\pm$ 3100). Similarly, the screening of plasma from bursectomized–irradiated (Bx–X) birds for the presence or absence of Ig appeared to be a sensitive index of humoral immunocompetence since no bird shown to be Aγ ever developed anti-NDV antibodies even after repeated inoculations with NDV.

The mortality rates of normal, sham operated-irradiated (SBx–X, STx–X), T cell deficient (Tx–X) and Aγ birds after a single intranasal inoculation of NDV_{Plag} are reported in Table I. It can be seen that Aγ chickens were extremely susceptible since none survived inoculation with $10^{4.8}$ ELD_{50} (embryo lethal dose) or more. Similarly, T cell deficient birds were more susceptible to NDV than were control-irradiated or normal birds. Clinically, Aγ and control birds developed ND characterized by the acute onset, usually 8–10 days after inoculation, of weakness, paralysis and progressively more severe myoclonic spasms which frequently ended in death within 48 hr. In T cell deficient birds, ND differed clinically; partial weakness and paralysis were more typical while myoclonic spasms were rarely seen. More than one third of dying T cell deficient birds became so weak and paralyzed that they starved and were euthanized after failing to show signs of recovery within 4 or 5 days.

We were unable to detect any significant differences in the kinetics and levels of HI antibodies between the three groups capable of elaborating humoral immune responses (Cx–O, STx–X and Tx–X).

The increased susceptibility of T cell deficient birds to NDV suggested that cell mediated immunity may afford some measure of protection from lethal NDV infection. Accordingly, we attempted to induce such immunity in Aγ birds with live vaccine (Bl strain) as well as BPL-inactivated NDV. The

TABLE I. DIFFERENTIAL SUSCEPTIBILITY OF NORMAL AND IMMUNODEFICIENT CHICKENS TO VARYING DOSES OF NDV_{Plag} INOCULATED INTRANSALLY.

Groups[a]	Mortality[b] after intranasal inoculation of $\log_{10}$ ELD_{50}					
	4.8	5.8	6.8	7.8	8.8	All doses
Normal	0/4	1/5	—	—	4/30	5/39
Irradiated control[c]	0/9	0/9	—	0/4	0/5	0/27
T cell deficient[d]	2/4	1/4	2/3	7/10	3/4	15/25
Agammaglobulinemic[e]	24/24	—	—	—	6/6	30/30

[a] Ten to 22 wk-old chickens.
[b] As number dying/number inoculated.
[c] Sham operated, sublethally X-irradiated (750R) at hatching.
[d] Thymectomized sublethally X-irradiated (750R) at hatching.
[e] Bursectomized sublethally X-irradiated (750R) at hatching.

TABLE II. RELATIVE PROTECTION AGAINST LIVE NDV_{Plag} CHALLENGE GIVEN BY DIFFERENT IMMUNIZATION PROCEDURES IN NORMAL AND IMMUNODEFICIENT CHICKENS.

Groups[a]	Immunization procedure[b]	Mortality[c] after intranasal challenge of $\log_{10}$ ELD_{50}					
		3.8	4.8	5.8	6.8	7.8	All doses
Irradiated control	—	4/11	0/9	0/9	—	0/4	4/33
	Bl	0/10	—	—	—	0/5	0/15
	BPL	0/5	—	—	—	—	0/5
T cell deficient	—	—	2/4	1/4	2/3	7/10	12/21
	Bl	—	—	—	—	0/5	0/5
Agammaglobulinemic	—	17/18	24/24	—	—	—	41/42
	Bl	0/5	—	—	5/5	—	5/10
	BPL	3/5	5/5	—	—	—	8/10
	NDV_{Plag}[d]	10/15	—	9/9	—	—	19/24

[a] See Table I.
[b] See text.
[c] As number dying/number inoculated.
[d] These birds had survived primary in inoculation of $10^{1.8}$ ELD_{50} or less.

latter was injected im in complete Freund's adjuvant and a booster injection given in saline im 3 wk later. "Immune" birds were challenged in with one of several doses of live NDV_{Plag} 3 wk after the booster injection of BPL-NDV or 3 wk after inoculation with the live vaccine strain. In all instances, the immunization procedures induced HI responses in all control-irradiated and T cell deficient birds. Some Aγ birds which resisted low doses of NDV_{Plag} were also rechallenged with higher doses. As can be seen in Table II, all control-irradiated chickens receiving the Bl vaccine strain 3 wk before challenge with NDV_{Plag} were protected regardless of the challenging dose. Similarly, T cell deficient birds were protected against a challenge which killed 7/10 nonimmune birds.

In Aγ birds, we could demonstrate some resistance against a small challenging dose of $10^{3.8}$ ELD_{50} following all three immunization procedures. Thus 12/25 immune Aγ birds resisted this challenge whereas only 1/18 Aγ birds has ever survived a similar but primary challenge. However, regardless of the immunization procedure in Aγ birds, protection could not be demonstrated against a stronger challenge. It is clear nevertheless that "immune" Aγ birds were less susceptible to NDV_{Plag} since 12/44 survived $10^{3.8}$ ELD_{50} or more whereas only 1/42 nonimmune Aγ birds was able to resist a similar challenge.

Discussion. These studies document the marked susceptibility of immunodeficient birds to an otherwise relatively avirulent strain of NDV. This susceptibility could not be ascribed to neonatal total body X-irradiation since the mortality rates in normal and neonatally irradiated sham operated chickens were 5/39 and 4/50 respectively over a wide dose range. All Aγ birds inoculated with $10^{4.8}$ ELD_{50} NDV_{Plag} or more succumbed and only 1/18 which received $10^{3.8}$ ELD_{50} survived.

Birds which were T cell deficient were capable of elaborating HI antibodies in a manner which did not differ from that of control birds. Nevertheless, 15/25 T cell deficient birds died following inoculation with $10^{4.8}$ ELD_{50} or more NDV_{Plag}. Although some of the groups tested involved few birds, the data clearly suggest a dose-related susceptibility. It is likely therefore that T cells are involved in initiating or helping to initiate immune responses against NDV. It is possible for instance that some anti-NDV antibodies are qualitatively different in T cell deficient and normal chickens; and that such differences might not be detectable in the HI assay. The protective role of T cell immunity is further suggested by the partial protection induced in Aγ birds immunized with avirulent NDV Bl, BPL-inactivated NDV or low doses of live NDV_{Plag}. However, this protection was limited to birds challenged with $10^{3.8}$ ELD_{50} and no immune Aγ bird survived a greater challenge.

In the analysis of the mechanisms of host resistance to NDV one must also consider nonspecific mechanisms such as interferon and activated macrophages which could also be disturbed in immunodeficient chickens. To our knowledge, these mechanisms have not been studied in these experimental models. Similarly, early patterns and rates of virus replication and dissemination in immunodeficient birds may differ widely from those seen in normal birds so that the detectable immune responses may provide relatively little protection if they are expressed after vital target organs have become affected. In this respect we have observed 50% mortality (12/24) in normal birds injected im with $10^{8.8}$ NDV_{Plag} when only 4/30 succumbed after in inoculation of the same dose. Moreover, in both im and in inoculated normal and T cell deficient birds which died, the HI responses were not distinguishable from those observed in survivors.

There are two recent reports of primary viral infections in immunodeficient chickens. When comparing these studies to our own, one should naturally keep in mind potential differences in the strains of birds used and their relative degree of immune deficiencies as well as the nature of the infective agent. Nevertheless, it is of interest that regardless of the infective agent used both humoral and cell mediated immunity influence the course of the disease. Indeed, in the studies of fowlpox infection, the mortality after a standard inoculation in normal, B cell deficient and T cell deficient birds was 0, 18 and 37% respectively, whereas all birds with combined B and T cell deficiency succumbed to the infection (12). When resistance to avian influenza virus was studied, the mortality in normal, B cell deficient and T cell deficient birds was 36, 84 and 48% respectively (13).

Precise definition of the mechanisms of resistance to NDV and of the pathogenesis of the disease awaits more specific assays of cell mediated immunity as well as a more detailed analysis of the qualitative and quantitative aspects of the humoral and nonspecific responses to this virus.

Summary. In order to assess the mechanisms of host resistance to Newcastle disease virus (NDV), the susceptibility of young adult normal, T cell deficient and agammaglobulinemic chickens to an avirulent live vaccine (Bl) and a mesogenic strain of NDV was studied. All animals, regardless of immunological status resisted the vaccine strain. Most normal birds resisted mesogenic NDV, however T cell deficient birds were much more susceptible and agammaglobulinemic chickens were extremely susceptible. There was no difference in the kinetics and levels of hemagglutination-inhibition activity of plasma between normal, control-irradiated and T cell deficient birds ncr between dying and surviving birds. Agammaglobulinemic chickens could be partially protected against an otherwise lethal challenge following immunization with avirulent NDV, low doses of mesogenic NDV inoculated in-

tranasally or im injection of β-propriolactone inactivated NDV mixed in complete Freund's adjuvant. The possible mechanisms for this protection together with the relative roles of humoral, cell mediated and non-specific immunity are discussed.

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Received April 4, 1974. P.S.E.B.M. 1975, Vol. 148.