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The Immunomodulatory Role of Calf Thymus Extract on Humoral and Cell Mediated Immune Response in Chicken Vaccinated Against New Castle Disease Virus

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Abstract: The effect of administration of calf thymus extract (CTE) with protein concentration of 1.8mg/ml was studied using two groups of day old layer chicks. Both the groups were vaccinated with 'F' and 'R2B' strains of New Castle Disease Virus (NDV) vaccines on 7th day and 8th week respectively. One group was administered with 1.8mg of thymic proteins intraperitoneally, one week prior and one week after each vaccination. The other group remained as vaccinated control group. Humoral and cell mediated responses were evaluated 15 days after each vaccination. Intraperitoneal administration of calf thymus extract markedly and significantly increased the antibody titres against NDV, serum globulin level, and percentage of lymphocytes in the blood. In addition thymus extract resulted in definite and significant cellular immunopotentiation.

Key words: Calf thymus extract, Immune response, new castle disease virus

Introduction

Newcastle disease (ND), a viral disease of domestic birds, still exists as an enzootic in different parts of the world, presenting a variety of clinical forms ranging from a mild to a fatal fulminating illness. The severity being determined primarily by the strain of the infecting virus and the level of immunity in the host. In spite of regular vaccinations, severe outbreaks often occur in areas of intensive poultry production, which is reasoned mainly to breakdown in immunity. This has prompted for introduction of variety of immunomodulators to restore the immunity.

The thymus is an important organ which plays a crucial role in the formation of the lymphoid structures in the prenatal and early postnatal period of life and orchestrating the lymphoid system throughout the life. Various studies with thymus extract have got remarkable immunomodulatory effects. The thymic extract restored the depressed functions of humoral and cellular immune systems in alloxan diabetic mice (Hadzija *et al*., 1987). It also enhanced the blastogenic response of peripheral blood lymphocytes to phytohaemagglutinin (PHA) and concavalin (conA) mitogens in chicken (Murthy and Ragland, 1992). Thymus extract has been found to enhance the antibody titers in birds vaccinated against infectious bronchitis virus, infectious bursal disease virus (Barbour *et al*., 1998).

Keeping in view the aboe immunomodulatory role of thymus extract and deadly nature of New Castle disease, the present study was designed to evaluate the immunomodulatory role of CTE in chicken vaccinated against NDV.

Materials and Methods

Source of birds: A total of 40, day-old layer chicks (BV-300) were obtained from Venkateshwara Hatcheries limited, Hyderabad, India. They were maintained on a balanced feed and water *ad libitum*.

Vaccine: The Lentogenic(F)and Mesogenic(R2B)strains of NDV vaccines were procured from 'Ventri Biologicals', Venkateshwara Hatcheries Limited, Hyderabad, India.

Preparation of Calf thymus extract (CTE): The Calf thymus extract was prepared as per the method employed by Nikitenko *et al.* (1984) with slight modifications. Briefly, 100 gms of calf thymus tissues were collected in sterile phosphate buffer saline (PBS) (pH 7.2), washed thoroughly with PBS. It was well triturated and subjected to Teflon homogenization. The extract was collected and diluted 1:10 by PBS. The prepared samples remained for two hours at room temperature, and then heated at 60°C in a water bath for 30 mins. The same was further boiled for two minutes, passed through whatman filter paper No. 2. The extract was autoclaved for an hour at 120°C. The protein estimation in the thymus extract was done by the method of Lowry *et al.* (1951) and was adjusted to a conc. of 1.8 mg/ml.

Experimental design: A total of 40, day-old commercial layer chicks were divided into two groups, test group with 25 chicks and control group with 15 chicks. Both groups were vaccinated with 'F'strain of NDV on 7th day by intraocular and intranasal routes where as 'R2B' strain of NDV by subcutaneous route in the 8th week. The chicks in the test group were administered with CTE @ 1.8 mg/chick/day, by Intra peritoneal (I/P) route for one week prior and one week after each vaccination. The control group remained as just vaccinated group, without administration of CTE.

Sampling: Blood without anticoagulant for serum separation was collected as well as heparinized blood (20 IU of heparin per one ml of blood) was collected from wing vein from each bird after 15th day of each vaccination from both the test as well as control groups.

Haemagglutination Inhibition (HI) test: The micro-test method described by Allan and Gouch (1974) was used for detection of H1 titers from serum samples collected on 15th day post immunization of birds.

Serum total proteins and globulin concentration: Total proteins and globulin concentration in the serum were estimated by modified Biuret and Dumas method (Dumas, 1971) using "Diagnostic reagent kit for in vitro determination of total proteins and Albumin in serum", supplied by span diagnostics limited, Surat, India.

Percentage Lymphocyte Count: Different leukocytic count was carried out as per Nambiar (1960) to find the percentage of lymphocytes in the blood.

Dinitrochlorobenze (DNCB) test: To study the cell mediated response, Cutaneous hypersensitivity test was performed as per Hari Babu *et al.* (1993). This test was performed on 15th day after each vaccination. Briefly the method includes 0.05 ml of 2 % DNCB in acetone was applied slowly drop by drop on the skin of neck region of birds. The solution was made to evaporate quickly by gently blowing and thereby preventing the solution to run down the neck region. The thickness of the skin at 0,24 and 48 hrs after application of DNCB was taken using vernier calipers.

Statistical analysis: The data were expressed as the Mean(SE comparison between two groups were made by the student 't' test.

Results

Haemagglutination-Inhibition (HI) titres: The results of HI are presented (Table 1).The mean log HI titres after primary vaccination with 'F' strain on 7th day were 2.705(0.05 in the treatment group, which received both vaccination and CTE administration. The titres in control group were 2.003 (0.06 which were only vaccinated but not administered with CTE. After second vaccination with 'R2B'strain,the titre values were in increasing trend in treatment group with 2.955 (0.39). The titer values remained almost same in control group as in primary vaccination with mean log titer values of 2.053 (0.08).The results in the present study indicated that there was significant increase of NDV antibody titres in thymus extract treated group ($P<0.01$) when compared to control groups.

Serum total proteins and globulin levels: The serum total protein and globulin level in birds after each vaccination are presented (Table 1). There was significant rise ($P<0.01$) in serum total proteins and globulin concentration in test group compared to control after both I and II vaccinations.

Dinitro chlorobenzene (DNCB) test: Twenty four hours after challenge, the area where DNCB was applied, was warm, hyperemic and diffusely oedematous and thickened. The surface presented a granulated appearance due to swelling of the feather follicles. The results were highly significant ($P<0.05$) (Table 2) in test group compared to control group indicating CTE has profound effect cell mediated immunity.

Percent lymphocyte count: The control group had lymphocyte counts of 57.17 (2.39) and 58.4 (3.21) per cent during 'F' and 'R2B' vaccinations respectively, while the test group had an increased mean lymphocyte count of 62.39 (3.33) per cent and 65.0 (1.2) per cent during 'F' and 'R2B' vaccinations respectively.

Discussion

In the orthodox scheme of T-cell ontogeny in mammals and birds migrant stem cells colonize the thymus and differentiate to become cortical thymocytes that give rise to medullary thymocytes, and these further differentiate into peripheral T-cells (Weissman, 1973). Thus, in order to fulfill the potential of the thymus as a contributing component of the immune system, it must recruit or be colonized by intrinsic stem cells (Le Douarin, and Jotereau, 1986). This process of colonization by precursor cells is an important prerequisite for intra thymic T-cell differentiation.

HI titres showed higher humoral immunopotentiation to NDV in the CTE administered group than in the control groups. The work on monitoring of immune levels in birds against NDV treated with CTE is very much scanty, however with the available literature, these results were in accordance with results of Mohammed *et al.* (1995) and Barbour *et al.* (1998), who recorded highest humoral immunopotentiation to NDV using calf thymus extract and thymulin hormone respectively.

The mechanism of this enhanced humoral immunity

Table 1: Different parameters in birds after vaccinations with "F" and 'R2B' strain of NDV

	I Vaccination		II vaccination Parameter	
	Control	Test	Control	Test
Total Proteins (gm%)	3.15[a]±0.06	5.36[b]±0.26	3.18[a]±0.01	5.85[b]±0.29
Albumin (gm %)	1.34±0.13	1.58±0.01	1.29±0.10	1.63±0.05
Globulin (gm %)	1.97[a]±0.05	3.77[b]±0.255	1.95[a]±0.21	4.22[b]±0.28
HI Titre	2.003[a]±0.06	2.705[b]±0.05	2.053[a]±0.08	2.935[b]±0.39
Lymphocyte (%)	57.17 ±2.39	62.39±3.33	58.4±3.21	65.00±1.2

Note: * Values are mean ±SE. ** Those with same superscripts do not differ significantly at P< 0.01

*** Values of HI titre are the mean of log10 of HI titres values ± SE

Table 2: Mean skin thickness (mm) of birds with DNCB test after each vaccination

Time of measurement	I Vaccination		II vaccination	
	Control	Test	Control	Test
0 hour	0.163±0.01	0.162±0.03	0.165±0.01	0.165±0.04
24 hour	0.208[a]±0.05	0.269[b]±0.03	0.214[a]±0.41	0.347[c]±0.03
48 hour	0.236[a]±0.03	0.308[b]±0.38	0.256[a]±0.03	0.381[c]±0.05

Note: Values are mean ± SE. Those with same superscripts do not differ significantly at P< 0.01

could be due to the effect of the immunopotentiators, administered intra peritoneally, on thymic cells, thus raising the thymus dependent humoral antibody responses (Okamoto *et al.*, 1993). It has been well established that thymic hormones like thymulin in the thymus extracts, stimulates lymphocyte differentiation (Brand *et al.*, 1977) , and enhances proliferative effect in mixed lymphocyte reactions (Cohen *et al.*, 1975), probably resulting in increased antibody production, thus enhancing the HI titer values in the CTE treated group.

The serum samples showed an increased level of total proteins in the CTE treated groups compared to the control group, which may be due to the stimulation of thyroid hormones by the crude thymus extract resulting in physiological hyperthyroidism (Abdel Fattah *et al.*, 1999). The increased total serum proteins enhanced the general health status of the birds contributing significantly to the immunity of the birds and also markedly without any mortality of the birds during the entire experiment period. Our results were consistent with Nikitenko *et al.* (1984), who found that thymus homogenate increases total blood proteins in calves and piglets.

The test group had relatively high concentration of serum globulins over the control groups, the globulin concentration increased in the test group after second vaccination which is also one of the most probable reasons for increase in the HI antibody titres against NDV and results were concurrent with Mohammed *et al.* (1995) and Abdel Fattah *et al.* (1999) who have recorded a significant increase in total proteins, particularly the serum globulins in thymus extract administered chickens.

This significant cell mediated immunopotentiation could be due to the ability of the thymus extract to induce the expression of variety of T-cell differentiation markers and to enhance T-cell functional activities *in vivo* and *in vitro* (Bach *et al.*, 1975), to increase mature T-lymphocytes (Parent *et al.* 1994) and to function as an immunomodulator by exerting control on cytokine production by peripheral blood mononuclear cells (Safieh Garabedian *et al.* 1993) and the ability to induce lymphocyte maturation (Kook and Trainin, 1974) and to increase lymphocyte differentiation (Brand *et al.*, 1977). In CTE treated groups, there was considerable reactivity of NBT (56.4%) compared to control groups (25.1%), which indicated the maximum level of non specific immune response in treated group.

There was no available literature on NBT reduction assay on birds administered with CTE, however there were reports which have indicated the effect of NBT assay as a measure of nonspecific immunity. Park *et al.* (1968) stated that intensity of NBT reduction roughly correlated with bactericidal activity. The results obtained for the phagocytic activity in CTE treated groups were in accordance with Mohammed *et al.* (1995) who has shown a significant phagocytic activity in CTE treated chicken.

The increase in phagocytic activity of phagocytes in CTE treated group could be due to the ability of the thymus extract to act as an immunomodulator by exerting control on cytokine production by peripheral blood mononuclear cells. (Safieh Garabedian *et al.* 1993).

The CTE treated birds, showed a significant increase in total leukocytic count over the control group, which was in accordance with many workers (Mohammed *et al.*, 1995; Barbour *et al.* 1998 and Abdel Fattah *et al.*, 1999) who have found that thymus extract and thymic hormones increases the total leukocytic count in chicken.

There was a very significant increase in number of lymphocytes in CTE treated groups, when compared to the control group, which is one of the probable reason for over all immunopotentiation and this could be due to the fact that thymic hormones activates T-cell rosettes (Wara and Ammann, 1975) and they enhance the differentiation, maturation and proliferation of lymphocytes.

The present study has formed as an unique application part of CTE for assessing the immunomodulatory effect in chicken vaccinated with NDV and treatment has resulted in consistent immunopotentiation of the humoral antibody response, cell mediated delayed hypersensitivity reaction.

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