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THE ROLE OF CELL MEDIATED IMMUNITY IN PROTECTION OF CHICKENS CHEMICALLY DAMAGED BURSA AGAINST NEWCASTLE DISEASE

(With 7 Tables)

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دور المناعة الخلوية في حماية ذات كيس فيبريسي المثبط كيميائياً ضد مرض النيوكاسل

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فـي دراسة لحماية الكتاكيت ضد مرض النيوكاسل الخالية من المسببات المرضية تم تقسيم ١٦٠ كتكوت إلي أربع مجاميع. تبين إن حقن مادة السيكلوفوسفاميد قد قضي على رد الفعل المناعـي بالأجسـام المضـادة كمـا هـو موضح بنتائج اختبارمنع التلازن الدموي. أثبتت الكتاكيت المحصنة والخالية من الأجسام المناعية القدرة على ضد العدوى بفيروس النيوكاسل تـم إثـبات رد الفعـل المناعـي باختبار منع التلازن الدموي كذلك اختبار التحدي بالعترة الضارية.

SUMMARY

160 SPF chicks were divided into 4 groups to study the mechanism of protection against Newcastle disease (ND) virus. Cyclophosphamide treatment of 18 days old emberyonated chicken SPF eggs depleted the humeral antibody response as shown by HI test .SPF chickens after humeral antibody depletion were found to be still protected against ND challenge after being vaccinated. Cell mediated immune response to ND vaccination which is indicated by lymphocyte blastogenesis and macrophage presented by challenge.

Key words: *Newcastle, chicken, bursal disease, vaccine, immunology.*

INTRODUCTION

Newcastle disease (ND) is an infectious disease of chickens and turkeys characterized by lesions in the respiratory tract, viscerals organ and brian, causing moderate to sever mortalities in susceptible flocks.

ND causes great economic losses due to the high mortality rate, reduction of meat and drop in egg production (Bisural and Narril, 1954). Intensified vaccination programe as the methode of choice for the controle of ND Partadiredgo *et al.*, (1979). Bursa of fabricious is the primary lymphatic main organ of poultry responsible for B-cell proliferation, hence destruction of this organ revealed a drawback effect of the humeral immune response in chicken.

Destruction of this organ takes place if the birds are subjected to infection with infectious bursal disease (Allan *et al.*, 1972) or chemical reagents like cyclophosphamide substance. Several works had been done with cyclophosphamide substance such as by Fadly *et al* .. (1987).

This work was planned to study the role of cellular immune response in protection against ND after suppression of humeral immune response by cyclophosphamide substance.

MTERIALS and METHODS

1- SPF chicks Eggs:

160 specific pathogen free eggs "SPF" were used for test application of cyclophosphamide "obtained from koomshiem, fayoum, Egypt"

2- Vaccine:

live ND vaccine lasota strain with a titer $10^{6.5}$ EID / ml used for vaccination via the drinking water. The vaccine obtained from Intervet® and tittered by neutralization test before vaccination.

3- Newcastle virus virulent strain:

VVNDV " 10^8 EID 50/ml " was used for challeng obtained from Vet. ser Vac. Res. Institute. Cairo

4- Cyclophosphamide:

"$C_7 H_{15} C_{12} N_2 O_2 P. H_2O$" obtained from sigma and used for depletion of B- lymocyte.

160 eggs "SPF" of 18 day old were obtained, in which 80 eggs inoculated with cyclophosphamide "3 mg / embryo" and 80 eggs non inoculated. Incubation of the inoculated and non inoculated eggs was carried out till hatching 80 chicks from inoculated eggs and other 80 chicks from non inoculated eggs were obtained and divided into 4 groups each of 40 chicks.

The first group was treated with cyclophosphamide and vaccinated with living ND vaccine, the second group vaccinated with living ND vaccine Hitchner B_1 strain "via eye drop at seven day post hatching" vaccine only, while the third group was treated with

cyclophosphamide only and the fourth group left without any treatment as control.

Each bird from vaccinated groups "1&2" was vaccined 21 days after hatching with living attenuated Newcastle vaccine lasota strain "via drinking water"

10 random blood samples were collected from each group at 7,14,21,28,35, and 42 days post vaccinations, 5 of them were taken for estimation of humeral immune "haemagglutination inhibition test", while the other 5 samples were used for evaluation of cell mediated immune response using lymphocyte blastogenesis and machrophage activity test.

Haemagglutination inhibtion test "HI test": it was carried out according to the method described by Majujabe and Hitchner "1977".

Evaluation of cell mediated immune response:

a- Lymphocyte blastogenesis: it was carried out according to Lucy (1974) and modified by Charles *et al* (1987). Evaluation of the test using MTT was carried out according to Mosramn (1983) and the results of the test was expressed as Delta optical density.

b- Mactophage activity test:

The test was done according to Barry & Joh *et al* (1988) and modified by El- Enbawy (1990).

Phagocytic percentage:

The Phagocytic index was done according to Richandsum and Smith (1981) and determined as follows:

$$\text{Phagocytic \%} = \frac{\text{Total No. of phagocytes which ingest more than 2 candida}}{\text{Total No. of phagocytes which ingest}}$$

NDV. challeng test:

10 chicks were chosen randomly from each group 7,14 and 35 days post vaccination and subjected to challenge test with 0.5 ml of VVNDV strain containing 10^8 EID 50 / ml.

The virus was titered by neutralizing test before challenge. The birds were observed for 15 days post challenge.

Birds died within this period were collected and subjected to detailed post mortem examination.

RESULTS

Results obtained are recorded in Table 1 –7.

DISCUSSION

This work was planned to investigate the role played by the cellular immunity in protection of chicken against NDV. The obtained results recorded that no haemagglutination Ab_s titer could be estimated in cyclophosphamide treated and vaccinating "group 1" table (1) compared with non cyclophosphamide treated and vaccinated group "group 2" table (1) wich recorded a low Ab titer "2.50". on week post vaccination reaching peak "8.08" three weeks post vaccination.

The obtained results denoting the effects of cyclophosphamide on depletion of B-lymphocytes, the results agreed with Lerman and Weidanz (1970) and Sperrs *et al* (1978). Istifanuse *et al* (1985) stated that birds treated with cyclophosphamide failed to senthesize detectable amount of Ab_s and they referred this results to suppressive effect of cyclophosphamide and atrophy of bursa of fabricious.

Controversially, progressive increase in the cellular and macrophage activity results of lymphocyte transformation test expressed as Delta optical denisty (table 2) revealed that maximum values were recorded 14 days post hatching in all groups, however the magnitude of the value was noticed when the test was applied specifically using NDV. as an Ag comparing with the use of phytohemagglutination "PHA" as mitogen. Obtained results revealed non noticeable differences between group 1&2 when the peak values recorded were "0.135" and "0.130" for both groups.

On the other hand, more assertion for the former results were obtained after evaluation of the macrophage activity. (tables 3&4), maximum phagocytic activity expressed as percentage and index recorded parameters were " 75.7%" and "72.3%", " 0.47" and " 0.46" for both groups respectivity. Results of protection percentage against the VVNDV reflect the immunological status of the expermintal chickens (tables 5,6,7) maximum protection 100% was recorded percentage for group (2) 14&35 days post hatching whereas the recorded percentage for cyclophosphamide treated and vaccinated group "group 1" at 3,14,35 days were 50%, 80% and 20%. The obtained results summarize the possible role played by cellular immune response in protection of humeral immune response as result of the cyclophosphamide treated. This finding comes in agreement with those of Jones *et al* (1992) and Chackal *et al* (1999) where they stated in similar work that other immune mechanism should play a principle role of protection against

TRTV infection in turkey after using cyclophosphamide to suppress the humeral immune response.

The results agreed also with those of Caporal *et al* (1978)& Schmidt and Massable (1974) where they expressed the role of local immune response for protection against some other infection in birds.

Table 1 : The average log. 2 of HI titer to NDV in chickens.

Group	7 days	14 days	21 days	28 days	35 days	42 days
1	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	2.50	5.66	8.09	7.05	6.35
3	0.0	0.0	0.0	0.0	0.0	0.0
4	0.0	0.0	0.0	0.0	0.0	0.0

Group 1: treated with cyclophosphamide and vaccinated with living NDV.
Group 2: vaccinated with living NDV vaccine only.
Group 3: treated with cyclophosphamide only.
Group 4: non treated & non vaccinated (control).

Table 2: Evalution of cell mediated immune response by lymphocyte transformation expressed by delta optical density.

DPV	7 days		14 days		21 days		28 days		35 days		42 days	
Group	PHA	NDV	PHA	NDV	PHV	NDV	PHA	NDV	PHA	NDV	PHA	NDV
1	0.095	0.120	0.122	0.140	0.135	0.165	0.112	0.145	0.090	0.110	0.065	0.080
2	0.098	0.117	0.117	0.135	0.130	0.160	0.110	0.148	0.081	0.108	0.052	0.078
3	0.010	0.116	0.090	0.012	0.010	0.017	0.008	0.013	0.008	0.011	0.011	0.011
4	0.011	0.014	0.009	0.015	0.015	0.012	0.011	0.014	0.012	0.015	0.010	0.015

DPV = day post vaccination
PHA = phytohaenagglutiuation

Table 3: Evaluation of cell mediated immune response by macrophage activity using Candida albicans expressed phagocytic percentage.

Group	7 days	14 days	21 days	28 days	35 days	42 days
1	54.1	75.7	62.5	50.7	40.9	35.2
2	56.2	72.3	64.5	49.2	43.1	38.1
3	18.2	12.2	16.4	16.9	12.9	10.4
4	12.7	15.2	19.3	14.5	16.5	11.3

Table 4: Evaluation of cell mediated immune response by macrophage activity using Candida albicans expressed phagocytic index.

Group	7 days	14 days	21 days	28 days	35 days	42 days
1	0.35	0.45	0.47	0.35	0.30	0.27
2	0.36	0.46	0.46	0.36	0.32	0.20
3	0.010	0.08	0.06	0.10	0.11	0.08
4	0.08	0.07	0.09	0.09	0.10	0.07

Table 5: Protection percentage for chickens 7 days post vaccination

Group	Total No. of chickens	No. of dead	No. of Protected	Percentage %
1	10	5	5	50
2	10	5	5	50
3	10	10	0	0.0
4	10	10	0	0.0

Table 6: Protection percentage for chickens 14 days post vaccination

Group	Total No. of chickens	No. of dead	No. of Protected	Percentage %
1	10	2	8	80
2	10	0	10	100
3	10	10	0	0.0
4	10	10	0	0.0

Table 7: Protection percentage for chickens 35 days post vaccination.

Group	Total No. of chickens	No. of dead	No. of Protected	Percentage %
1	10	8	2	20
2	10	0	10	100
3	10	10	0	0.0
4	10	10	0	0.0

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