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ROLE OF B CELLS, T CELLS AND MACROPHAGES IN THE IMMUNE RESPONSE OF CHICKS TO NEWCASTLE DISEASE VIRUS*

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SUMMARY: Two groups of chicks were immunosuppressed by thymectomy or bursectomy, followed by antithymus serum and antibursal serum treatment. The third group of chicks were immunosuppressed by cyclophosphamide and antibursal serum treatment. Carrageenan treatment was undertaken with the fourth group of chicks to impair the macrophage function. These chicks were vaccinated at weekly intervals starting from the 10th day to 59th day of age. After assessing the humoral and cell mediated immune response, the chicks were challenged at weekly intervals to assess the protection per cent to Newcastle disease virus. It was found that in thymectomised vaccinated chicks the protection was 64.2%, in bursectomized vaccinated chicks the protection was 52.4% and the balance of 16.6% protection appeared to be due to the interaction of B cells, T cells and macrophage in chicks vaccinated with La Sota strain of Newcastle disease virus.

Keywords: Newcastle disease virus, Carrageenan

INTRODUCTION

There are reports that B cells and T cells play a key role in the mechanism of protection against Newcastle disease (Perey *et al.*, 1975). B cell and T cell deficient models were also experimentally produced (Matsuda and Bito, 1973; Perey *et al.*, 1975) to study the immune responses to Newcastle disease virus (NDV) in chicks. Macrophages either alone or in co-operation with T lymphocytes were shown to be involved in the activation of B lymphocytes by thymus independent antigens (Chused *et al.*, 1976; Blease, 1975; Biozzi *et al.*, 1975). In our study, an attempt has been made to find out the role of T cells, B cells and macrophages in normal and immunosuppressed chicks vaccinated with La Sota strain of NDV.

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MATERIALS AND METHODS

Chicks

Day-old chicks received from Poultry Research Station (PRS), Madras were reared in isolation in chick brooders and were free from adventitious infections. These chicks were given chick mash and water *ad libitum*.

Ducks

Six eight-month-old Khaki Campbell ducks obtained from PRS were used for the production of antibursal serum (ABS) and antithymus serum (ATS).

NDV La Sota Strain

The NDV La Sota strain obtained from the Institute of Veterinary Preventive Biological Products, Pune, India, having a titre of $10^{6.74}$ EID_{50}/ml was used.

ATS and ABS

The ATS and ABS were produced as per the procedure described by Jaiswal *et al* (1981) and Mishra and Jaiswal (1984).

Colloidal Carbon

A suspension of colloidal carbon (Gunture, Wagnes Pelikan wrke, Hanover, Germany) in stoppered bottle was stored as stock solution of 64 mg/ml with little quantity of gelatin. The stock solution was melted in boiling water, thoroughly shaken and diluted to concentration of 16 mg/ml before use.

Carrageenan type II (CGN)

CGN (Sigma) essentially consisting of iota CGN was dissolved in 0.1 M phosphate buffered saline (PBS) (pH 7.3) and stored at a concentration of 10 mg/ml.

T Cell Deficient (Tx) Chicks

One hundred Tx chicks were experimentally produced by surgical thymectomy following the procedure of Herbert (1978) within 36 hr of hatch. These chicks were treated with 1 ml of 1:10 dilution of ATS subcutaneously (SC) daily for 4 consecutive days. A hundred control chicks were shamthymectomized (STx) and were given 1 ml of PBS, SC for 4 consecutive days.

B Cell Deficient (Bx) Chicks

Seventy Bx chicks were experimentally produced by surgical bursectomy following the procedure of Herbert (1978) within 36 hr of hatch. In addition, all the Bx chicks were inoculated with 1 ml of 1:10 dilution of ABS, SC for 4 consecutive days. Seventy control chicks were shambursectomized (SBx), within 36 hr of hatch and were inoculated with 1 ml of PBS, SC for 4 consecutive days.

CGN Treatment of Chicks

The procedure described by Murthy and Ragland (1984) was followed.

Colloidal Carbon Clearance Test

The method described by Stuart *et al* (1978) was followed. The relative amount of carbon in blood samples were estimated in photoelectric calorimeters.

Experimental Design

The immunodeficient chicks were divided into three groups. The first group contained 48 Tx chicks, 48 STx chicks and 48 control chicks. The second group contained 48 Bx chicks, 48 SBx chicks, 48 Cy treated chicks and 48 control chicks. The third group contained 18 CGN treated chicks, 18 vaccinated control and 18 unvaccinated control chicks. The chicks of group I and II were vaccinated with La Sota strain of NDV intranasally on 10 , 17, 24, 31, 38, 45, 52 and 59th day of age. Chicks of group III were vaccinated with La Sota strain of NDV intraperitoneally at 24th day of age.

In-vitro *Assessment of Immune Response*

The B cell response was assessed by the microhaemagglutination inhibition (MHI) test, following the procedure of Timms and Alexander (1977) and by the modified passive haemolytic plaque forming cell (PFC) assay, as per the procedure described by Cunningham and Szenberg (1968). T cell response was assessed by the lymphocyte migration inhibition (LMI) test, as per the procedure of Timms and Alexander (1977). LMI assay was performed using 3 to 4 X 10^6 sensitised spleen cells/ml. Then 0.1 ml of 1:5 dilution of sonicated NDV La Sota strain was used as antigen per ml of RPMI-1640 medium. The average migration area in the presence or absence of antigen was then compared and the percentage of inhibition calculated. The modified lymphocyte cytotoxicity test was followed to assess the T cell response as described by Ganger and Williams (1968). Suspensions of lymphocytes from the spleen of immune and non immune chicks were prepared in Eagles minimum essential medium containing 10 per cent foetal calf serum. The embryo fibroblast cells were used as target cells. Spleen cells were adjusted to 1:100 target, effector ratio. In this experiment, the effector cells were sonicated and the resulting supernatant fluid was placed in tubes containing chick embryo fibroblast cells and incubated at 37°C for 4 hrs. The quantum of live fibroblast cells left over after the cytotoxic action of the lymphocytic extracts is indicated by 3_H thymidine uptake which will get incorporated only in the DNA of live cells. The role of macrophages in immune response was studied by carbon clearance test as per the procedure described by Stuart *et al* (1978).

Immune Response to Challenge

One hundred CMD_{50} doses of virulent NDV, obtained from the Institute of Veterinary Preventive Medicine, Ranipet, was used to challenge the vaccinated chicks of groups I, II and III at weekly intervals after each vaccination along with unvaccinated controls.

RESULTS AND DISCUSSION

When overall mean MHI titres of Tx group of chicks was compared with STx group by *t* test, there was a marginal reduction in the MHI titres and the differences in HI response was statistically significant ($P<0.05$) (Table 1).

This indicates that HI response through B cell activity is still marginally affected in Tx chicks. Similarly, when the PFC response of chicks was compared with STx chicks, it was found to be highly significant ($P<0.01$) (Table 1). This proves that PFC assay is more sensitive in eliciting the influence of thymus on humoral response than the MHI test. Since there is no corresponding work with regard to NDV as per the available literature perused, there is no possibility of correlating these results.

Table 1. Comparison of overall mean HI titre and mean PFC value in shamthymectomised and thymectomised chicks.

Treatment	Mean HI Titre		Mean PFC Value	
	Pre-vaccinal	Post-vaccinal	Pre-vaccinal	Post-vaccinal
STx Group	1.75	41.13 ± 2.98^a	135	$1,279 \pm 10.0^a$
Tx Group	1.25	33.08 ± 2.72^b	129	$1,054 \pm 10.1^b$

STx - Shamthymectomised; Tx - Thymectomised.
Row means with different superscripts differ significantly.

The results of overall mean LMI per cent revealed that the LMI response was higher in SBx group of chicks than in Bx and Cy-treated group of chicks ($P<0.01$) (Table 2).

Table 2. Comparison of mean toxicity percentage, mean percentage of LMI values in shambursectomised, bursectomised and cyclophosphomide treated chicks.

Group of chicks	Overall mean percent of cytotoxicity	Overall mean percent of LMI values
SBx	40.2 ± 1.26^a	62.7 ± 1.30^a
Bx	28.9 ± 0.34^b	58.2 ± 1.30^b
Cy	29.6 ± 0.78^b	53.5 ± 0.60^c

SBx - shambursectomised; Bx - bursectomised; Cy -cyclophosphamide treated
Row means with different superscripts differ significantly.

The results of LMI response agree with the findings of Perey *et al.*, (1975) and Timms and Alexander (1977). The fact that the LMI response in Cy-treated chicks got depressed when compared with SBx group of chicks corroborates the findings of Lerman and Weidanz (1970) and Dietrichi and Dukor (1967). The results of overall mean cytotoxicity per cent similarly indicate that there is significant difference between the SBx group of chicks on one hand and Bx as well as Cy treated group of chicks on the other (Table 3). However, the difference in cytotoxicity response between Bx as well as Cy treated group of chicks is not appreciable when compared within them. Therefore, these results imply that both B and T cells are required to induce protection against NDV, confirming the findings of Cheville and Beard (1972) and Mishra and Jaiswal (1984). The effect of CGN treatment on macrophages has been assessed by carbon clearance test (Table 3).

Table 3. Comparison of mean phagocytic index value on 3rd. and 10th. day post-vaccination in normal vaccinated, normal unvaccinated and Carrageenan (CGN) treated vaccinated chicks.

	Normal vaccinated	Normal unvaccinated	CGN-treated vaccinated
Phagocytic index value	10.75 (6)	8.25 (6)	8.85 (6)

Number in parenthesis indicates the number of chicks used in the colloidal carbon clearance test

It is observed that the overall mean phagocytic index was low in CGN treated vaccinated chicks when compared with normal vaccinated chicks indicating that the macrophage function has been affected by CGN treatment.

When the challenge results were statistically analysed by normal deviation test, it was found that the protection level in STx group was 100 per cent and in Tx group only 64.2 per cent which was highly significant ($P<0.01$) (Table 4).

Table 4. Comparison of protection percent of normal vaccinated and immunosuppressed vaccinated and unvaccinated control chicks.

Group I			Group II				Group III		
Tx	STx	Control	Bx	SBX	Cy	Control	NV	CGN	Control
64.2^b (51)	100.0^a (51)	0.0^c (51)	52.4^b (19)	100.0^b (19)	37.3^b (19)	0.0^c (19)	100.0^a (12)	50.0^b (12)	0.0^c (12)

Numbers in parenthesis indicate number of chicks used in the challenge test
Figures with different superscripts differ significantly.

The marginal decrease in protection correlates well with the marginal decrease in humoral response in the Tx group. This signifies that the thymus has got a marginal control in inducing protection against NDV. Similarly in the SBx group, the protection per cent was 100 per cent and in Bx chicks and Cy treated chicks, the protection percentage were 52.4% and 37.3% respectively. These findings are in agreement with results of Mishra and Jaiswal (1984) and Cheville and Beard (1972). The Cy has been found to depress the humoral response (Prasad 1978) and therefore the lack of protection could be correlated to its action. Both bursectomy and Cy treatment have affected the T cell function; the former marginally and the latter more significantly and this may be the reason for the limited protection afforded by these groups. In the CGN treated group, only 50 per cent of the chicks survived on challenge against 100 per cent protection in normal vaccinated chicks. This shows that CGN has affected macrophage function in a

significant way as assessed by carbon clearance test. These results already point out that any impairment to any of these three cell populations would definitely result in the impairment of protection percentage to NDV. It is not possible to have an arithmetical delineation of the individual role played by T cells, B cells and macrophages in protection against NDV. Being a biological system, there could be interaction between these three cells both synergistically as well as antagonistically. It is found that in bursectomized vaccinated chicks where the T cells are intact, the protection was found to be 52.4%, in thymectomised vaccinated chicks where the B cells are intact, the protection was found to be 64.2% and the interaction of B cells, T cells and macrophages appear to be responsible for the resultant excess of 16.6% of protection in chicks immunised with La Sota strain of NDV.

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RINGKASAN

PERANAN SEL B, SEL T DAN MIKROFAJ DALAM GERAK BALAS IMUM TERHADAP VIRUS PENYAKIT NEWCASTLE

Dua kumpulan anak ayam telah diimunotindas menerusi timektomi dan bursektomi, diikuti oleh perlakuan serum antitimus dan serum antibursa. Kumpulan anak ayam ketiga telah diimunotindas menerusi perlakuan siklofosfamid dan serum antibursa. Perlakuan Carrangeenan telah dijalankan pada kumpulan anak ayam keempat untuk merosakan fungsi makrofajnya. Anak ayam tersebut telah divaksinkan pada selang satu minggu bermula daripada umur sepuluh hari sampai umur 59 hari. Selepas menilai gerak balas imun humoral dan berantarakan sel, anak ayam tersebut dicabar pada selang satu minggu untuk menilai peratus perlindungan terhadap virus penyakit Newcastle. Apa yang didapati ialah dalam anak ayam tervaksin tertimektomi, perlindungan ialah 64.2%, dalam anak ayam tervaksin terbursektomi, perlindungan ialah 52.4% dan baki perlindungan 16.6% itu nampaknya disebabkan oleh saling tindakan sel B, Sel T dan makrofaj dalam anak ayam yang divaksin dengan strain La Sota virus penyakit Newcastle.