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Cell Mediated Immune Response to Newcastle Disease Vaccine (*Lastoa strain*) in Chicks

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Abstract: This research was carried out at the Department of Microbiology, University of Agriculture Faisalabad, Pakistan in order to study Macrophage Migration inhibition Factor produced by lymphocytes. The M1F activity of control samples without any plasma sample showed an average migration of macrophages with 410.23 μ. Migration of macrophage inhibition activity in single vaccinated broiler chicks when compared with control macrophage migration, the macrophage migration inhibition% activity ranged from 14. 29 to 35.7% with the average of 28.57%. Further it was observed that maximum MIF activity in this group did not exceed to 35.7% 4 broilers showed 35.7%, 3briolers showed 28.57%, 2briolers showed 21.43% and one showed 14.29% MIF (%) activity in 10 broiler chicks. Migration of macrophage inhibition activity in boostered vaccinated broiler chicks when compared with control macrophage migration, it was investigated that ten-broiler chicks showed MIF per cent activity after double vaccination ranged from 28.57 to 42.86%. Further it was found that 3 broilers showed 28.57% 1 broiler showed 32.13%, 2 broilers with 35.7% and 4 broilers showed 42.86% MIF activity. The results obtained during present investigations revealed that Lasota vaccine strain of ND induces MIF 28.57% after 14 days of first vaccination and to obtain a better immune response, a double vaccination, 7 days after the single one is necessary.

Key words: New castle disease, lasota strain, broilers, cell mediated immune response

INTRODUCTION

Poultry farming have become remarkably a profitable industry recently. To keep birds healthy advanced prophylactic techniques are adopted which help in eradication and control of the infectious and contagious diseases. Among these Newcastle disease is one of the most important hazards, which can only be controlled through prophylactic measure. The vaccine used to protect birds from disease should be properly evaluated for assessing their efficacy. Cell mediated immunity and humoral immunity together play an important role to provide Protection against disease[1].

Immunity due to microbial infections involves both humoral (B-lymphocytes/antibody) and cell mediated (T-lymphocytes/lymphokines). Both these responses are essential for complete protection against infections Chandraseker *et al*.,[2]. Cell Mediated Immunity (CMI) is produced against infections caused by micro-organisms which enter and grow within tissue cells, e.g., Newcastle disease virus, salmonella and Brucella and Mycobacterium tuberculosis Duguid *et al*.,[3] . The cell-mediated immune system includes several cell types and their products. Macrophages present antigen to T-Lymphocytes. T-cell receptors and various hormones recognize the antigens and a specific T-cell clone become activated and begin to proliferate as reported by Jawetz *et al*.,[4]. It is generally recognized that impairment of cell mediated immunity results in an increased susceptibility to virus infections as cited by Lodmell *et al*.,[5]. T-lymphocytes after coming in contact with antigen release factors collectively known as lymphokines. These factors can be measured in vitro. Macrophage Migration Inhibition Factor (MIF) is among one of these factors, which prevents, in vitro, migration of macrophage as described by Outtridge[6].

The recognition of cellular immunity as an important mechanism in protective immunity and in causing tissue damage in auto allergic diseases has promoted the development of numerous *in vitro* assays for cellular immunity. These cellular assays are considered, *in virto*, correlates with cell-mediated immunity. In recent years, several lines of evidence suggest that cell mediated immunity plays an important role in defense against viral infections as revealed by Habasha *et al*.,[7].

To determine the cell mediated immune response in chicks against ND vaccines, macrophage migration inhibition factor assay was selected because it is simple, easy to perform and facilities were available in the department. The aim of this project was to study MIF

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production by, Lymphocytes, from broiler chicks, vaccinated with Newcastle disease vaccine (Lasota strain), to assess CMI response and to assess the relationship between the two vaccinated and non-vaccinated chicks.

MATERIALS AND METHODS

Experimental animals: Thirty, day-old broiler chicks were procured from Olympia chicks, Faisalabad kept at the experimental poultry farm, University of Agriculture, Faisalabad. The chicks were provided feed and water and were divided into three groups. Group A comprised of 10 chicks, vaccinated with commercial Newcastle Disease (ND) vaccine (Lasota strain) through drinking water at the age of 14 days. Blood samples were collected aseptically 11 days after vaccination and lymphocytes were separated. Group B comprised of 10 chicks, vaccinated with LaStoa vaccine strain of ND virus at the age of 14 days through drinking water and a booster dose was given after 7 days through drinking water. Blood samples were collected after 14 days post booster vaccination. Group C comprised of 10 chicks and was kept as control to obtain peritoneal macrophages.

Vaccine: Freeze-dried ND vaccine (LaSota strain, Pliva Zagreb Croatia Batch NO. 2414082) containing 1000 doses was purchased from the local market and placed at 4°C till use. The vaccine was suspended in 10 mL were added to 500 mL water, making a standard dose for 30 chicks. Out of 20 chicks, 10 chicks were revaccinated after 7 days of first vaccination in the same way.

Preparation of macrophage Migration Inhibition Factor (MIF): MIF was prepared following the method of Harrington and stastny[8] with slight modification as follows .Birds from both groups A and B were vaccinated with NDV (Lasota strain) at the age of 14 days and after 14 days of first vaccination; 1-2 mL of blood was drawn from wing vein. Heparinized blood of the chicks was allowed to stand in syringes for 2 hrs at room temperature for the separation of plasma and erythrocytes. Plasma containing lymphocytes were separated by turning the needle at 90° and plunging the syringes till erythrocytes layer. Plasma was suspended in Rosewill Park Memorial Institute (RPMI-1640) medium in 1:1. RPMI 1640 was supplemented with 5% heat-inactivated bovine fetal calf serum (flow lab.) and (antibiotics 100 u ml-1 penicillin and 100 ug u mL-1 streptomycin). The plasma was then dispensed into culture tubes and 1 mL/one dose of Newcastle disease vaccine virus (Lasota strain) was added into it. The tubes were incubated at 41C for 48 hrs in an incubator. After 48 hrs of incubation, the suspension was spun in a refrigerated centrifuge at 1500 rpm for 10 minutes. After centrifugation, the supernatant was separated, filtered through a membrane filter (Millipore APD=0.45 μ and the filtrate was used as MIF.

Macrophage preparation: Chicken peritoneal macrophages were harvested using a Sephadex stimulation method following Qureshi and Miller[9] and Trembicki *et al.*,[22].

Briefly, a single injection of 3% sephadex G-50 (sigma) suspension in sterile saline (0.75%) was injected into the peritoneal cavity of birds at the rate of 1 ml per 100 g body weight. The birds were slaughtered after 42 hrs and the abdominal skin was disinfected with alcohol and incision through the skin was made. The skin was reflected by blunt dissection and the upper and lower parts of the chicken were covered with surgical towels. A 30-50 mL of sterilized Hank's Balanced Salt Solution (HBSS) was injected into the peritoneal cavity with the help of a Siliconized plunger and a gentle massage of abdomen was applied.

The Peritoneal Exudate Cells (PEC) suspension was collected from the peritoneal cavity into the sterilized plastic tubes and centrifuged at 1500 rpm for 10 min at 4°C. Immediately, after centrifugation, the supernatant was discarded and pellet of PEC was obtained. Micrometrical studies of macrophage were conducted including the average size and the total number of macrophages present per ml in PEC suspension as described by Benjamin[11].Pooled PECs were mixed in 1:1 with 2x RPMI-1640 having 5% obvine fetal calf serum for migration inhibition assay and were mixed with 0.2% agarose suspension.

Macrophage migration inhibition test: Migration of macrophages was measured by using agarose droplet Method (Harrington and Stastny[8] in 24 well disposable polystrin, titration plates (Limbro Space Saver, Flow Lab) with slight modification. One drop of 0.8% (w/v) agarose in water was dispensed onto the middle of each well with a 22 gauge blunt tipped needle immediately after heating to melting temperature. Care was taken to touch each drop to form a hemisphere. Wells were kept warm by gently placing on a light box until the agarose drops were dried to provide a firm bond between the microtitration plate surface and cells suspension droplet to be added later. Care was taken to avoid excessive drying of this pre-coated drop.

Immediately after drying of the agarose pre-coat, one drop of PEC suspension in agarose was placed over the

Table 1: Counting of macrophage in PEC suspension

Area of hemocytometer	Total No. Counted	Total No. of macro-pages per cu/mm	Total macro-phages/ml of PEC
Left upper chamber (LU)	16	$3.7x\ 10^3$	$3.7xl0^6$
Left lower chamber (LL)	21		
Right lower chamber (RL)	20		
Right upper chamber (RU)	15		

Table 2: Macrophage migration inhibition after single vaccination with LaSota at the age of 14 days in broiler chicks

Ocular mm reading (S.d*)	Experimental migration in (μ.)	Inhibition of migration (μ.)	MIF-activity (μ) (%)
9	263.7	146.53	35.7
11	322.3	87.93	21.43
9	263.7	146.53	35.70
9	263.7	146.53	35.70
10	293.0	117.23	28.50
10	293.0	117.23	28.50
12	351.6	58.63	14.29
11	322.3	87.93	21.43
9	263.7	146.53	35.70
10	293.0	117.23	28.57
Average	293.00	117.23	28.57

Macrophage migration in control = 410 .23 m (14 S.D.)

Table 3: Macrophage migration inhibition in the boostered vaccinated broiler Chicks 7 days after single vaccination with LaSota in broiler chicks

Ocular mm reading (S.d*)	Experimental migration in (u)	Inhibition of migration (u.)	MIF-activity (u) (%)
8	234.4	175.83	42.86
10	293.0	117.23	28.57
8	234.4	175.83	42.86
8	234.4	175.83	42.86
9	263.7	146.53	35.70
9.5	278.40	131.83	32.13
10	293.0	117.23	28.57
10	293.0	117.23	28.57
8	234.4	175.83	42.86
9	263.7	146.53	35.70
Average	262.24	147.99	36.07

Macrophage migration in control = 410.23 m (14 S.D)

precoated area of each well and the wells were cooled down at 4°C for 5 min. Each well containing a drop of PEC suspension was filled with 1 mL of RPMI-1640 containing 0.25 mL of MIF, taking care to avoid excessive drying of droplets.

Migration of PECs from the agarose droplet was measured after 24 hrs by determining the distance from the edge of the droplet to the periphery of the macrophage migrated. An inverted microscope with ocular micrometer mounted in eye piece was used for this measurement, Harrington and Stastny[8], Habasha *et al.*,[7], Agrawal and Reynolds[12]. The calibration was made at 2.5x objective of inverted microscope. The stage and ocular micrometer were used for this purpose. The zero division of ocular micrometer was adjusted with the zero of stage micrometer division for the calibration of ocular micrometer.

Migration Inhibition Factor (MIF) from the plasma: Straw-colored plasma was processed for each sample in order to determine the MIF activity after sensitization of leukocytes with the LaSota strain of a Newcastle disease virus. A light straw colored plasma was obtained after centrifugation and filtered through membrane filter (Millipore APD=0.45u).Chicken Peritoneal Exudates Cells (PEC) suspension was harvested with a final volume of 25 mL (HBSS) in sterilized siliconized glass beaker.

Determination of size and number of macrophages: A single drop of PEC suspension was smeared on the clean glass slides and fixed with ethanol (Absolute). Later the slides were stained with Giemsa's stains and observed under oil immersion objective of the already calibrated microscope. It was investigated that varying size of macrophages, some were engorged with sephadex particles, were present in each field. The average size of the unengored macrophages was 13±0.5 μ in diameter with a well demarcated nucleus at the center. The PEC suspension when processed for the determination of total number of macrophages per ml of suspension using a haemocytometer, it was observed that 3.7x106 cells were present per ml of suspension. The total amount of PEC suspension was centrifuged in plastic tubes under refrigerated temperature (4°C). The packed volume of Macrophages was obtained in one ml of HBSS and washed two times in the HBSS.

An inverted microscope was calibrated at 2.4 x power objective and it was found that one small division of

ocular micrometer equals to 29.3 u. Total distance of macrophage migration from the edge of Agarose droplet was measured separately for each sample after 24 hrs of incubation (41°C).

The linear distance of migration was measured from edge of droplet to the perimeter of migration by adjusting 0 division of ocular micrometer with the edge of the droplet. The migration in single vaccinated samples and boostered samples were measured and percentages were calculated with the control as follows.

$$\%M = \frac{Migration\ distance\ in\ the\ presence\ of\ antigen}{Migration\ distance\ in\ the\ control} X100$$

The results come in percentage macrophage migration. The percentage macrophage migration inhibition was determined as follows.

%age inhibition = 100 - percentage macrophage migration. Generally more than 20% inhibition in the presence of MIF shows a significant macrophage migration inhibition activity.

RESULTS

The M1F activity of control samples without any plasma sample showed an average migration of macrophages with 410.23 μ. Migration of macrophage inhibition activity in single vaccinated broiler chicks when compared with control macrophage migration, the macrophage migration inhibition per cent activity ranged from 14. 29 to 35.7% with the average of 28.57%. Further it was observed that maximum MIF activity in this group did not exceed to 35.7% 4 broilers showed 35.7%, 3 briolers showed 28.57%, 2briolers showed 21.43% and one showed 14.29% MIF (%) activity in 10 broiler chicks. Migration of macrophage inhibition activity in boostered vaccinated broiler chicks when compared with control macrophage migration, it was investigated that ten-broiler chicks showed MIF per cent activity after double vaccination ranged from 28.57 to 42.86%. Further it was found that three sample showed 28.57%, one sample showed 32.13%, two sample with 35.7% and 4 sample showed 42.86% MIF activity.

When the paired t-test was applied on the average MIF activity between single and boostered vaccinated groups it was highly significant ($p<0.01\%$). The correlation coefficient of MIF activity between single and boostered vaccinated group of broiler chicks was found to be highly significant with r=0.99 in terms of average macrophage MIF activity. It means that with boostered vaccination MIF activity was increased with LaSota strain.

DISCUSSION

The percent macrophage migration inhibition, 14 days post single vaccination with LaSota strain of ND vaccine at an age of 14 days ranged from 14.29 to 35.7% with mean percent migration inhibition 28.5%. These results are in agreement with Kumar *et al.*,[1] studying humoral and cell mediated immune responses in chicks vaccinated with ND vaccine reported 32.3% leukocyte migration inhibition after the end of second weeks which was 21.3% at the end of first week and considered as positive. The primary response continued rising till 5th week and the declined. In the present study, macrophage migration inhibition technique was used instead of leukocyte migration inhibition test. The results are partially in agreement with that of Chubb *et al.*,[13] who reported that MIF in birds sensitized against infectious bronchitis virus is related to sensitizing dose of virus. In the present study, a standard does of vaccine virus was used. The results obtained during present investigation are not in accordance with finding of Brundage *et al.*,[14] who carried out research trail to study cell mediated response in a porcine enterovirus infection in piglets and found a weak and localized cell mediated immunity measured by indirect leukocyte migration inhibition test .This controversy may be due to the difference in antigen used or may be the host.

The results of the present study are coincided with the finding of Cameron and Rensturg[15], Cameron *et al.*,[16], Habasha *et al.*,[7], who reported that MIF was closely associated with immunity. MIF was best with undiluted antigen and undiluted lymphocyte supernatant and better response was observed with live vaccines than inactivated vaccines. In the present study, diluted antigen and lymphocyte culture supernatants were used. The finding of present study are in agreement with Results of Chaturuedi and Sharma[17,18] who investigated A moderate cellular response in first week and a sharp increase during second and third weed and a sharp increase during second and third week after infection in calves with salmonella Dublin . In the present study response was measured 14 days after immunization.

The Results obtained during present investigation are also in close vicinity with the finding of Romia *et al.*,[19] who reported a marked cell-mediated immune response, manifested by significant increase in the percentage of MIF in human against strongyloidiasis. The percent macrophage migration inhibition of chicks given with Lasota strain of ND vaccine determined 14 days, post -double vaccination ranged from 28.75 to 42.86% with mean 36.07% migration inhibition. The finding investigated during present study are not in accordance with results obtained by Kumar *et al.*,[1] and Agrawal and

Reynolds[12] who reported a non-significant increase in percent leukocyte migration inhibition test in chicks vaccinated with LaSota vaccine. The difference may be due to time of second vaccination; antigen and technique used in the experiments. The finding obtained during present study are in line with result obtained by Onaga and Ishii[20] who reported a high leukocyte migration inhibition factor in thrice inoculated chicks with Eimeria tenella even 3 weeks after last inoculation. The results of present investigation are also in agreement with the finding of konopa[21] who studied cell -mediated immunity in experimental listeriosis in different animals and reported that the strongest and the most durable cell-mediated response induced by subcutaneous injection of immunogenic strain followed after 14 days by oral infection with a virulent strain. Suzuki *et al*.,[22] also reported an enhance macrophage migration inhibition induced by sensitized T cells from mice treated with Toxoplasmosis lysate antigen and obioactin.

It was for the 1st time that the cell mediated immune response was assessed using Macrophage Migration Inhibition Factor (MIF) activity in broiler chicks vaccinated with LaSota Strain of Newcastle Disease (ND) virus. Present work has open up future avenues using the MIF activity as response variable. Further research project may be planned to study the kinetics of maternal antibody response with respect to MIC. However, provisions shall also be given to compare the CMI response from vaccinated and carrier state of the bird against Newcastle disease virus infection.

REFERANCES

1. Kumar, K.U., S.K. Swamy and T.V. Reddy, 1988. Humoral and cell-mediated immune response in chicks vaccinated against newcastle disease. Ker. J. Vet. Sci., 19: 116-121.
2. Chandrasekar, S., R.A. Venkatesan, V.D. Padmanaban and P.R. Masiilamony, 1989. Nature of protective immunity in chicken against ranikht disease. Indian Vet. J., 66: 801-806.
3. Dugudi, J.P., B.P. Marmion and R.H.A. Swain, 1978. Medical Microbiology 13th Edn. English Language Book Society, London, 1: 144.
4. Jaweetze, E., L.M. Joseph, A.A. Edward, F.B. George, S.B. Janet and L.N. Ornston, 1989. Medical Microbiology 18th Ed. Appleton and Lange/SanMateco California, pp: 103-111.
5. Lodmell. D.L., A. Ninex, K. Hayashi and A.E. Notkins, 1973. Prevention of cell to cell of Herpes simplex virus by leukocytes. J. Exp. Med., 113: 706-720.
6. Outtridge, P.M., 1985. Veterinary Immunology. Academic Press Eondon, pp: 12.
7. Habasha, F.G., B.P. Smith, L. Schwartz, A. Ardans and M.R. Guerra, 1985. Correlation of macrophage migration inhibition factor and protection from challenge exposure in calves vaccinated with salmonella typhimurium. J. Vet. Res., 46: 1415-1421.
8. Harrington, J.R., T. John and P. Stastny, 1973. Macrophage migration from an agarose dropt: Development of micro method for assey of delayed hypersensitivity. J. Immunol., 110: 752-759.
9. Qureshi. M.A. and E. Miller, 1991. Comparison of macrophage fictioninseveral Commercial broiler lines. Poult. Sci., 70: 2094-2101.
10. Trembicki, K.A., M.A. Qureshi and R.R. Dietert, 1984. Avian peritoneal exudates cells: A comparison of stimulation protocols. Develop. Comp. Immunol., 8: 395-402.
11. Benjamin, M.M., 1978. Outline of Veterinary Clinical Pathology. 3rd Ed. The Iowa state University Press Ames. Iowa, U.S.A.
12. Agrawal, P.K. and L.R. Donald, 1991. Evaluation of the cell mediated immune response of chicken vaccinated with Newcastle disease virus as determined by the under agarose Leukocyte migration-inhibition technique. Avian Dis., 35: 360-364.
13. Chubb, R., V. Haynh and R. Bradley, 1988. The induction and control of delayed type hypersensitivity reaction included in chicken by infectious bronchitis virus. Avian Path., 17: 371-383.
14. Brundag, L.J., J.B. Derbyshire and B.N. Wilkie, 1980. Cellmediated responses in a porcine enterovirus infection in piglets. Canadian J. Comp. Med., 44: 61-69.
15. Cameron, C.M. and J.J. Van Rensburg, 1975. Inhibition of macrophage migration in Salmonella immunity Onderst. J. Vet. Res., 42: 15-24.
16. Cameron, C.M., J.J. Van Rensburg and M.M. Engeldrecht, 1976. Relation between macrophage migration inhibition and immunity to Brucella aborts in guinea pigs. Onderst. J. Vet. Res., 43: 175-184.
17. Chaturvedi, G.C. and V.K. Sharman, 198l. Cell-mediated immunoprotection in calves immunized with rough salmonella Dublin Brit. Vet. J., 137: 421-430.
18. Chaturvedi, G.C. and V.K. Sharma, 198 I. Cellular and humeral immune responses in calves experimentally infected with Salmonella Dublin. Zantralblatt fur Bakteriology Mikrobiology und Hygiene, 249: 494- 503. (Vide vet. Bull. Abst., 51: 7274).

19. Romia, S.A., A.E. Handoussa and E.M. MakhloufandEl-Malky, 1988. Duodenal immunoglobulins and. CMI response inhuman strongyloidiasis.J. Egypt Soc. Parasite., 18: 609-617.
20. Onaga, H. and T. Ishii, 1980. Leukocyte migration inhibition in chickens immunized with Eimeria tenella. Japanese J. Vet. Sci., 42: 345-351.
21. Konopa, M., 1984. Cell-mediated immunity in experimental Eisteriosis in animals. Wetrynaria, 41: 17-32. (Vide Vet. Bull. Abst., 56: 756).
22. Suzuki, N., K. Kikushima, T. Miyagami, I. Igarashi, H. Sakurai, A. Saito and H. Osaki, 1987. Modulator effect of Toxoplasma lysate antigen in mice experimentally infected with Plasmodium berghei, zentralblatt-fur- Bacteriology Microbiologic und -Hygiene, 264: 422-434. (Vide protozoological Abst., 12: 149).