A COMPARATIVE STUDY OF RESPIRATORY PHAGOCYTIC CELL ACTIVITIES IN LAYER CHICKS

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SUMMARY: A comparative study of avian respiratory phagocytes (ARPs) was conducted in four different strains of commercial layer chicks. The birds were raised to 12-14 weeks of age and were used as source of ARPs. Respiratory phagocytic cells harvested 42 hours post-inoculation of 3% Sephadex G-50 into the abdominal air-sacs were assayed for their viability, substrate adherence potential and percentage of macrophages in ARPs. The mean percentage values of viable cells after Sephadex G-50 stimulation were higher in groups B and D compared to groups A and C. Group D showed the maximum while group A the lowest value of substrate adherence potential of ARPs. Group B produced the maximum percentage of macrophages in ARPs. The results of present study suggest that different genetic strains vary in their phagocytic cell characteristics.

Key Words: Phagocytic cells, respiratory macrophages.

INTRODUCTION

Disease resistance against invading pathogens has two important aspects, i.e. cellular and humoral immunity. Cellular activity responsibles for phagocytosis and other aspects of specific and non-specific resistance are carried out by a diverse group of cells including microphages and macrophages (4).

Initially, the most significant research was conducted in mammalian species to understand the biology of phagocytic cells. However, with the introduction of new techniques to harvest the avian respiratory phagocyties (5), it was noted that respiratory exudate cells (AECs) were highly activated after stimulation. Different immunogens have been used, through both peritoneal and abdominal air sac routes to induce an exudate with an increased number of cells. These include lymphokines, sensitized T-lymphocytes, antigen-antibody complexes (11), bacterial endotoxin, *Pasturella mutocida* (7,16), *Salmonella typhimurium* (17) and a variety of chemicals such as Freund's incomplete adjuvant (5) and 3% Sephadex G-50 (1).

Based on the knowledge of cell biology and functions as well as the fact that such functions are influenced by genetic and species variations (2), the present study was conducted to compare the respiratory phagocytic cell characteristics in four genetic strains of commercial layer chicks.

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Table 1: Percentage of viable ARPs harvested from Sephadex G-50 stimulated and control layer chicks.

	Percentage of Viable Cells			
Treatment	Group A	Group B	Group C	Group D
Sephadex G-50	78.75 ± 3.75^{cd}	90.75 ± 4.75^{ab}	81.25 ± 3.75^{bc}	93.75 ± 2.75^{a}
Control	70.25 ± 2.25^{d}	89.50 ± 1.00^{abc}	69.50 ± 1.50^{d}	81.00 ± 1.00^{bc}

Mean values ± SE with no common superscripts are significantly different at $P \leq 0.05$.

MATERIALS AND METHODS

Chicks

Eighty, day-old layer chicks of four strains procured from different commercial layer brooding companies were raised to 12-14 weeks of age. These strains were randomly designated as group A, B, C and D. Each group comprised of twenty birds which were further subdivided into two subgroups, each having ten birds. The birds of one subgroup were stimulated by Sephadex G-50 through abdominal air-sacs while the others received normal saline and served as control.

Isolation of Avian Respiratory Phagocytes (ARPs)

The respiratory phagocytes were harvested using a Sephadex G-50 stimulation method modified from Ficken *et. al.* (5) and as adopted by Toth and Siegel (15). Briefly, the respiratory phagocytes were harvested 42 hours post-inoculation of Sephadex G-50 (3% suspension) and normal saline into the abdominal air-sacs of experimental and control birds respectively at the dose rate of 1ml/100mg of body weight (1). The phagocytes were collected by inserting a sterile pipette tip connected to a 50 ml syringe into the trachea and lavage fluid (PBS) was slowly injected into lungs and air-sacs until the air-sacs were *fully expanded.* The ARPs were collected in plastic tubes by aspirating the lavage fluid and centrifuged at 2000 rpm for 10 minutes. After centrifugation, the supernatant was discarded to obtain phagocytic cell pellet which was resuspended in RPMI-1640 growth medium supplemented with 5% heat inactivated bovine fetal calf serum and antibiotics (100 μ g/ml penicillin and 50 μ g/ml streptomycin).

Cell Viability Assay

The viability of ARPs was evaluated by trypan blue exclusion technique previously described (8). Viable cell concentration in the pooled ARPs samples was adjusted to 1×10^6/ml after counting with a haemocytometer.

Substrate Adherence Potential

Pooled ARPs from ten birds were used to determine the substrate adherence potential. One ml of 1×10^6 ARPs was added to each of the petridishes containing four coverships. After incubating at 41°C for one hour, the coverships were washed, fixed and stained. The number of adherent cells from four randomly selected fields was scored for each coverslip at 1.000 x microscopically (9). The results were expressed as mean adherent cells per sample.

Percentage of Macrophages in ARPs

The ARPs were allowed to adhere on glass coverships and 200 adherent cells per covership were classified by morphological criteria (6) at 1000 x to determine the percentage of macrophages in ARPs population.

Statistical Analysis

The data thus collected was subjected to statistical analysis and the treatment means were separated by Duncan's Multiple Range (DMR) test (14).

RESULTS AND DISCUSSION

The importance of comprehensive immune assessment has initiated the scientists to develop numerous *in vitro* assays for cellular immunity. Macrophages are the major cells, which play an important role in the cellular immunity by engulfing and destroying the invading pathogens (13).

The percentage of viable ARPs in Sephadex G-50 stimulated and control birds showed a statistically significant ($P \leq 0.05$) difference among different groups. The mean viable cell percentage in Sephadex G-50 stimulated and control groups ranged from 78.75±3.75 to 93.75±2.75 and 69.50±1.50 to 89.50±1.00 per cent respectively (Table 1). In Sephadex G-50, treated birds percentage of viable cells was significantly higher in group B and D while lowest in group A. In case of Sephadex G-50, treated birds Group C and D showed a significantly higher percentage of viable ARPs when

Table 2: Substrate adherence potential of ARPs harvested from Sephadex G-50 stimulated and control layer chicks.

	Number of Cells Per Microscopic Field			
Treatment	Group A	Group B	Group C	Group D
Sephadex G-50	47.97 ± 1.04^{c}	56.13 ± 1.76^{b}	59.09 ± 1.41^{b}	67.34 ± 1.69^{a}
Control	13.16 ± 0.61^{f}	17.06 ± 0.79^{e}	15.03 ± 0.47^{ef}	26.69 ± 0.99^{d}

Mean values ± SE with no common superscripts are significantly different at P≤0.05.

compared with their respective controls. These results are inconsonance with (15) who reported the viability of exudate cells to be more than 90%. Aslam *et. al.* (2) also reported that different strains of commercial layer chicks showed a variable percentage of viable cells when stimulated by Sephadex G-50. In a study on turkey respiratory macrophages, the viability of cells had been reported as 95% (5).

The ARPs were allowed to adhere on glass cover-slips to determine the number of adherent cells per microscopic field. There was a significant difference among the groups in respect of substrate adherence potential. Group D showed the highest while group A, the lowest number of cells per microscopic field in Sephadex G-50 stimulated and control birds. The mean (±SE) number of adherent cells ranged from 47.97±1.04 to 67.34±1.69 in Sephadex G-50 stimulated groups whereas, in the control group it was between 13.16±0.61 to 26.69±0.99. All the groups showed a significant difference in adherence potential of Sephadex G-50 stimulated birds from their respective controls (Table 2). The results of present study are in line with Aslam (3) and Rauf *et. al.* (10) who conducted studies on four different strains of layer and broiler chicks respectively and reported that they differed among themselves in respect to substrate adherence potential. Mediator activated macrophages show increased stickiness and ruffled membrane motility. An increased macrophage adherence is a biologic effect of macrophage migration inhibition factor (12). The current study also strongly suggests that Sephadex G-50 stimulation significantly increases the adherence potential of ARPs.

The percentage of respiratory macrophages in adherent ARPs was determined. After Sephadex G-50 stimulation group B showed the highest while group C showed the lowest percentage of macrophages. All groups, after Sephadex G-50 stimulation showed higher percentage of macrophages in comparison to their respective controls. The mean (±SE) percentage of macrophages after Sephadex G-50 stimulation ranged from 74.50±1.13 to 88.06±1.14 while 28.06±0.68 to 44.94±1.15 per cent in case of control groups (Table 3). Trembicki *et. al.* (18) reported that peritoneal exudates from unstimulated animals contain 10^{6}-10^{7} resident macrophages and stimulation can fur-

Table 3: Percentage of macrophages in ARPs harvested from Sephadex G-50 stimulated and control layer chicks.

	Percentage of Macrophages			
Treatment	Group A	Group B	Group C	Group D
Sephadex G-50	87.00 ± 1.00^{a}	88.06 ± 1.14^{a}	74.50 ± 1.13^{c}	83.06 ± 1.93^{b}
Control	44.94 ± 1.15^{d}	35.81 ± 1.82^{e}	30.06 ± 0.66^{f}	28.06 ± 0.68^{f}

Mean values ± SE with no common superscripts are significantly different at P≤0.05.

ther increase the number of macrophages. The percentage of purified macrop-hages in ARPs, harvested from turkey was reported to be 95.7±5.9 per cent (5). These results are also strengthened by Aslam (3) who reported that Sephadex G-50 stimulated peritoneal exudate cells showed a significantly higher percentage of macrophages than cells from control groups.

In conclusion, the present study suggests that different genetic strains vary in their phagocytic cell characteristics, thus vary in their response to vaccine or natural contact with antigen. The present findings can be used as guideline to study ARPs in normal, diseased and immunomodulated birds to assess the direct effect of cytokines, antibiotics, vaccines toxins etc. Moreover, as various infectious diseases are quite prevalent in Pakistan both in layer and broiler chicken, apart from the other control measures selection of resistant breeds through such studies may be a major breakthrough for combating this alarming situation.

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