COMPARISON OF MACROPHAGE FUNCTION IN SEVERAL COMMERCIAL CHICKEN BROILER BREEDS

N.I. Chaudhary, H. Afzal, M. J. Arshad, M.K. Shafiq and M.T. Islam
Department of Veterinary Microbiology,
University of Agriculture, Faisalabad-38040, Pakistan

ABSTRACT

A study was conducted to establish a baseline profiles of various macrophage functions in four commercial chicken broiler breeds (local) designated as groups A, B, C and D. The experimental birds were between 4 to 6 weeks of age. Total number of peritoneal exudate cells per bird collected 42 hours after a single intraperitoneal injection of Sephadex G-50 were compared among these breeds, Groups B and C produced more macrophages than groups A and D. Macrophages from group B exhibited significantly higher and group A lower phagocytic activity for opsonized and unopsonized sheep red blood cells (SRBC). Macrophages from group A displayed significantly reduced bactericidal activity against unopsonized *Escherichia coli*. This study has thus demonstrated breed variations among the four commercial chicken broilers for mononucular phagocytic system functions.

INTRODUCTION

Macrophages arise from bone marrow stem cells, called promonocytes. The immediate progeny of these cells are monocytes which enter and remain in blood circulation for few days, then enter tissues as mature macrophages (Roitt, 1991).

Disease resistance has at least two important aspects i.e. it is both under humoral and cell mediated control. Most significant research directed toward understanding the biology of macrophages has been conducted in mammalian species. However, with the development of procedures to harvest chicken macrophages to perform studies on avian macrophage biology. Specifically, the experimental modulation of avian macrophage functions *in vitro* has become possible (Trembicki *et al.*, 1984). Macrophage monolayers established from this exudate on glass or plastic substrate has exhibited typical macrophage growth characteristics *in vitro*. Functionally, these Sephadex G-50 elicited macrophages were highly activated. They were capable of phagocytizing antibody coated or uncoated targets, possess bactericidal properties (Qureshi *et al.*, 1986), secrete enzymes and express Fc transferrin receptors and Ia molecules (Qureshi *et al.*, 1989).

Evidence from both avian and mammalian systems indicates that various macrophage functions are influenced by specific genetic differences between strains (Qureshi and Miller, 1991).

The present study was, therefore, conducted to establish baseline profiles of various macrophage functions in various local commercial chicken broiler breeds.

MATERIALS AND METHODS

Day old chicks of four different commercial chicken broiler local breeds were used as the source of peritoneal macrophages. The birds were then randomly designated as group A, B, C and D. In each group, 10 birds were kept as experimental and the other 10 served as controls and were kept under similar managemental conditions. The birds of 4-6 weeks of age were used as source of peritoneal exudate cells (PECs).

Isolation of Peritoneal Exudate Cells (PECs)

PEC were harvested using a Sephadex G-50 stimulation method as modified by Sabet *et al.* (1977) and previously described by Trembicki *et al.* (1984). A single injection of 3 per cent Sephadex G-50 was injected into the peritoneal cavity of each bird at the dose rate of 1 ml/100 g of body weight. The birds were slaughtered after 42 hours following injection and were surgically exposed.

From 20-30 ml of sterilized PBS containing sterile heparin (0.5 IU/ml)) were injected into the peritoneal cavity and the fluid was circulated well in the peritoneal cavity by prodding the anterior abdominal wall. From each birds, PEC suspension was collected into plastic tubes and spun at 1500 rpm for 10 minutes in a refrigerated centrifuge. After centrifugation, the supernatant was discarded to obtain PEC pellet.

Macrophage cultures

PEC from individual bird were resuspended in RPMI 1640 (Sigma and Co. USA) growth medium supplemented with 5 per cent heat inactivated bovine foetal calf serum and antibiotics (100 U/ml penicillin and 50 mg/ml streptomycin).

To determine the incidence of macrophages, total non-erythroid PEC from each of the bird were counted on a haemocytometer. One ml PEC suspension from each of the 10 birds per group was transferred into petri dishes containing four sterile round cover slips. Petri dishes were incubated at 41°C for one hour to allow macrophages adherence. Cover slips were washed with sterile saline to remove all non-adherent cells and other debris, fixed in methanol, stained with May-Grunwald-Giemsa, mounted on clean glass slides and approximately 200 adherent cells per cover slip were determined morphologically under microscope at 1000 x magnification (Lucas and Jamroz, 1961).

Sheep Red Blood Cell (SRBC) Phagocytosis Assay

The phagocytic activity of macrophages belonging to different breeds of chicken broilers was datermined using an in vitro SRBC phagocytosis assay as described by Qureshi *et al.* (1986).

Bactericidal Assay

The bacterial killing assay was performed as described in detail for bovines by Desiderio and Campbell (1983) and chicken macrophages by Qureshi *et al.* (1986).

The data thus collected were subjected to statistical analysis and the treatment means were separated by Duncan's Multiple Range (DMR) test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Incidence of adherent macrophages from Sephadex G-50 stimulated and control broiler birds was determined. The percentage of macrophages in the adherent cell population was higher in groups B and C than in the groups A and D. However, the results showed a considerable variation in percentage of adherent macrophages in PEC (Table 1). Mediator activated macrophages exhibited increased stickiness and ruffled membrane motility and it was noted that increased macrophage adherence was the biologic effect of macrophage migration inhibition factor (MIF) as discussed by Piessens *et al.* (1975).

Phagocytic ability of macrophages from different breeds was determined using opsonized and unopsonized sheep red blood cells (SRBCs). The phagocytic potential of group B was significantly higher than the rest of the groups. However, phagocytic activity was higher in groups B and C for unopsonized SRBC. All the groups except group C showed significantly higher phagocytic activity for opsonized than unopsonized SRBCs. The control birds showed a non-significant differences among themselves for both opsonized and unopsonized SRBC (Table 2). These results are in line with Arshad *et al.* (1996) who have reported that the phagocytic ability of macrophages increases significantly against opsonized SRBCs than unopsonized SRBCs. Golemboski *et al.* (1989) have found that early macrophages had more than 2 per cent of cells capable of phagocytizing unopsonized SRBCs, 36.5 per cent phagocytic for opsonized SRBCs compared with 60 per cent and 99 per cent, respectively, for late peritoneal macrophages obtained

Table 1: Incidence of glass adherent macrophages in Sephadex G-50 elicited PECs collected from different commercial broiler chicken breeds.

	Percentage of macrophages			
Treatments	Group A	Group B	Group C	Group D
Sephadex G-50	64.80b	77.56a	76.64a	61.52b
Control	23.27d	40.16c	28.47d	31.98cd

Table 2: Phagocytic potential of Sephadex G-50 elicited chicken macrophages isolated from different commercial broiler chicken breeds.

Treatments	Percentage of phagocytic macrophages			
	Group A	Group B	Group C	Group D
a. Opsonized				
Sephadex G-50	54.65c	80.82a	66.27b	71.31b
Control	16.27e	17.15e	13.35e	13.75e
b. Unopsonized				
Sephadex G-50	30.98d	64.36b	63.30b	47.73c
Control	16.81e	15.47e	16.89e	11.60e

Figures sharing at least a letter in common are statistically non-significant.

Table 3: Bactericidal potential of Sephadex G-50 elicited chicken macrophages isolated from different commercial broiler chicken breeds.

Treatments	Percentage of bacteria killed within 15 minutes			
	Group A	Group B	Group C	Group D
a. Opsonized				
Sephadex G-50	32.00abc	34.66ab	34.66ab	38.67a
Control	14.66efgh	25.33bcde	17.33efgh	18.66efg
b. Unopsonized				
Sephadex G-50	21.33def	32.00abc	24.00cde	30.66abc
Control	18.66efg	17.33efgh	16.00efgh	21.33def

Figures sharing at least a letter in common are statistically non-significant.

from female white leghorn chickens, 4 and 42 hours after injection of 3 per cent Sephadex G-50. This lack of phagocytosis of unopsonized SRBCs is caused by the lack of an appropriate cell surface receptor on early inflammatory macrophages. Phagocytic activity of Sephadex elicited PEC for latex particles or antibody sensitized SRBCs was found to be 99.5 to 100 per cent (Sabet *et al.*, 1977).

The process of phagocytosis is apparently mediated through the Fc receptors present on the macrophage surface (Chu and Dietert, 1988). Phagocytosis of unopsonized SRBSc by chicken macrophages appears to be an inducible property acquired during the *in vivo* activation of chicken macrophages or the *in vitro* activation of monocytes, (Neldon-Ortiz and Qureshi, 1992).

The effect of Sephadex G-50 stimulation on the bactericidal activity of peritoneal macrophages was determined by using an *in vitro* bactericidal assay (Desiderio and Campbell, 1983). A highly pathogenic Congo Red (CR+) phenotypic variant derived from *E. coli* strain was used in this assay. On statistical analysis in case of Sephadex G-50 treated birds, all the groups showed a non-significant difference among themselves against opsonized *E. coli*. However, group B showed a significantly higher bactericidal activity against unopsonized *E. coli* when compared with group A. Controls of all the four groups showed a non-significant difference in terms of bacteria killing against both opsonized and unopsonized *E. coli*. Group A and C showed significantly higher bactericidal activity against opsonized *E. coli* when compared with unopsonized *E. coli* (Table 3). These results are supported by Aslam (1994) who reported that the bactericidal potential of macrophages was significantly enhanced when *E. coli* were pre-treated with specific antibodies.

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