COMPARISON OF MACROPHAGE FUNCTION IN SEVERAL COMMERCIAL CHICKEN BROILER BREEDS

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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 mononuclear 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 Fe 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.

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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 determined 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).

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

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sephadex G-50</td>
<td>64.80b</td>
<td>77.56a</td>
<td>76.64a</td>
<td>61.52b</td>
</tr>
<tr>
<td>Control</td>
<td>23.27d</td>
<td>40.16c</td>
<td>28.47d</td>
<td>31.98cd</td>
</tr>
</tbody>
</table>
Table 2: Phagocytic potential of Sephadex G-50 elicited chicken macrophages isolated from different commercial broiler chicken breeds.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Opsonized</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Sephadex G-50</td>
<td>54.65c</td>
<td>80.82a</td>
<td>66.27b</td>
<td>71.31b</td>
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<tr>
<td>Control</td>
<td>16.27e</td>
<td>17.15e</td>
<td>13.35e</td>
<td>13.75e</td>
</tr>
<tr>
<td>b. Unopsonized</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sephadex G-50</td>
<td>30.98d</td>
<td>64.36b</td>
<td>63.30b</td>
<td>47.73c</td>
</tr>
<tr>
<td>Control</td>
<td>16.81e</td>
<td>15.47e</td>
<td>16.89e</td>
<td>11.60e</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Opsonized</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sephadex G-50</td>
<td>32.00abc</td>
<td>34.66ab</td>
<td>34.66ab</td>
<td>38.67a</td>
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<tr>
<td>Control</td>
<td>14.66efgh</td>
<td>25.33bcde</td>
<td>17.33efgh</td>
<td>18.66efg</td>
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<tr>
<td>b. Unopsonized</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sephadex G-50</td>
<td>21.33def</td>
<td>32.00abc</td>
<td>24.00cde</td>
<td>30.66abc</td>
</tr>
<tr>
<td>Control</td>
<td>18.66efg</td>
<td>17.33efgh</td>
<td>16.00efgh</td>
<td>21.33def</td>
</tr>
</tbody>
</table>

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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.

REFERENCES


