IN VITRO STUDIES ON PHAGOCYTIC ACTIVITY OF SEPHADEX G-50 ELICITED CHICKEN PERITONEAL MACROPHAGES

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ABSTRACT

Chicken peritoneal exudate cells (PEC) were induced by Sephadex G-50 injection and were used for studying their *in vitro* phagocytic activity towards sheep red blood cells (SRBC) and *Salmonella gallinarum*. The peritoneal macrophages were phagocytic to opsonised as well as to non-opsonised SRBC or *S. gallinarum*. The recognition and phagocytosis depended on time of exposure as well as the intensity of ligand molecules on the surface of phagocytic particles but was independent of the age of macrophage culture.

INTRODUCTION

Mammalian macrophages play an important role in the acquired immune responses as well as non-specific defense mechanisms. Macrophages process and present antigens to immunocompetent lymphocytes in association with class I and class II surface products of the major histocompatibility complex (Unanue, 1984; Weaver and Unanue, 1990). Macrophages also provide co-stimulatory activity for T-lymphocyte activation (Weaver and Unanue, 1990). The present knowledge is mainly based upon the *in vitro* studies of mammalian macrophages. The PEC, collected after stimulation of the peritoneal cavity with various irritants, are readily available source of the chicken macrophages for *in vitro* studies. These chicken peritoneal macrophages (PEM) have been studied for diagnostic procedure (Rose and Hesketh, 1974), phagocytic activity (Sabet *et al.*, 1977; Chu and Dietert, 1988) bactericidal and tumor-lysis (Qureshi and Miller 1990), monoclonal antibody production (Trembicki *et al.*, 1986), and genetic influences on the macrophage function (Qureshi *et al.*, 1988). Little information is available regarding factors affecting phagocytic activity of chicken peritoneal macrophages. The present study is designed to investigate factors affecting phagocytic potential of PEM to SRBC and *S. gallinarum*.

MATERIALS AND METHODS

Chicken Peritoneal Macrophages

Peritoneal macrophages were harvested from the cavity of white Leghorn chickens of haplotype MHC B (5 weeks old) by injecting Sephadex G-50 (Rose and Hesketh, 1974).

Perparation of Peritoneal Macrophages

The PEM were diluted to 2 x 10^6 mL in RPMI-1640. An aliquot of one mL of the cell suspension was transferred to a 35mm culture Petri-dish. The culture dishes were incubated at 39.6 C° with 5 % CO_2 for one hour. The culture dishes were washed with phosphate buffered saline (PBS) to remove non-adherent cells (Muhammad *et al.*, 1994). The adherent cells were subjected to study their phagocytic activity.

Measurement of Phagocytic Activity

Sheep red blood cells (SRBC) suspension was prepared from sheep blood and finally diluted in PBS to get 0.1 and 5 % suspension. The hyperimmune serum against SRBC as well as *S. gallinarum* were raised in poultry (Muhammad, 1993).

Anti-SRBC and anti-salmonella antibodies in the hyperimmune sera were monitored by a haemagglutination (HA) and bacterial agglutination assays, respectively (Alton *et al.*, 1971). The SRBC as well as *S. gallinarum* suspensions were opsonised (Chu and Dietert, 1988). The live bacterial suspension in RPMI-1640 medium (1.10^5/mL) without antibiotics was opsonised with one unit of anti-salmonella serum by overnight incubation at 4°C. The opsonised salmonella preparation was used for the phagocytic assay. The adherent cells of the PEC in 35 mm culture dishes were covered with 3 mL aliquot of either SRBC or *S. gallinarum* suspension in antibiotic free medium. The dishes were reincubated at 37°C with 5 per cent CO_2 for 30 minutes. The dishes were gently flushed with warm PBS at 37°C and stained with Giemsa's stain. The phagocytic and non-phagocytic cells were counted and the percentage of phagocytic cells was calculated.

The effects of degree of opsonization, phagocytic exposure time, nature of phagocytic particles and culture age of the macrophages on the phagocytic potential of chicken peritoneal macrophages were observed.

RESULTS AND DISCUSSION

The identification and phagocytosis of particles with some specific molecular moieties (ligands) is one of the main characteristics of macrophages. Like that of mammals, chicken PEM phagocytosed the opsonised and non-opsonized particles. It was further observed that increasing concentration of antibody to opsonized SRBC or *S. gallinarum* particles proportionately increased the percentage of phagocytic macrophages (Table 1). Increasing the antibody (opsonin) concentration might have increased the number of available Fc molecules on the particles which facilitates the movement of plasma membrane of macrophages around the particles and ultimately augments their phagocytic potential. These results are supported by Chu and Dietert (1988) who observed 100 per cent chicken PEM phagocytic when exposed to SRBC opsonised with 26 HA units of the opsonin. This kind of mechanism has been postulated for phagocytosis by mammalian macrophages (Adam and Hamilton, 1984; Cohn, 1987). Decreasing the concentration of opsonin reduces not only the percentage of phagocytic chicken PEM but also mitigates the number of particles phagocytosed per macrophage.

It was observed that 30-50 per cent of chicken macrophages were phagocytic. This phagocytic potential was reduced to 10-16 per cent when the dose of Sephadex G-50 used to harvest the PEM was doubled (Table 2). This suggested that phagocytosis independent to Fc receptor by chicken PEM may be mediated by receptors which might have decreased by increasing the dose of the irritant. These findings are in line to those reported by Ezekowitz *et al.* (1981) who observed that murine macrophages recruited by Bacillus Calmette-Guerin (BCG) were significantly less efficient in phagocytosing non-opsonised particles than those recruited by thioglycolate broth. BCG activates the macrophages during recruitment and induces a marked reduction in receptors responsible for phagocytosis of non-opsonised particles. Many other workers found similar type of receptors which can bind glycoprotein molecules having terminal mannose moieties. The density of such mannose specific receptors decreases in murine macrophages by *in vitro* exposure to a macrophage activating factor (MAF). The number of such receptor sites are substantially diminished in the later stages of macrophage activation (Adam and Hamilton, 1984). In the light of work on murine macrophages, it is concluded that phagocytosis of non-opsonised particles in chicken PEM could be mediated by mannose like receptors. However, the possibility of complement mediated phagocytosis can not be ruled out because like human macrophages, chicken PEM might be secreting complement components in *in vitro* culture (Whaley, 1980). Direct binding of such active complement components to SRBC could have contributed the percentage of chicken macrophages phagocytosing the opsonised or non-opsonised SRBC.

Table 1: Effect of opsonising antibody concentration on the phagocytic activity of chicken peritoneal macrophages

Antibody HA units	Phagocytic Macrophags (%)	Distribution of macrophages on the basis of number of RBC/macrophage (%)					
		1-2	3-4	5-6	7-8	9-10	>11
0.00	52.1	64.5	30.9	04.5	00.0	00.0	00.0
0.25	54.2	23.8	26.2	31.0	04.8	07.1	07.2
0.50	86.5	09.9	19.8	15.8	23.8	15.8	16.8
2.00	99.1	10.9	11.9	12.9	10.9	19.8	26.7

The 5% sheep red blood cells were incubated at 37°C with 0.25, 0.5 and 2.0 haemagglutinating (HA) units of anti-SRBC antibodies for 30 minutes. The opsonised SRBC were washed and resuspended in RPMI-1640 containing 10% foetal calf serum to give 5 % suspension. The monolayer of chicken peritoneal exudate macrophages (PEM) in each well was covered with either of these opsonised SRBC and incubated for 30 minutes for phagocytic interaction. Then each well was washed with distilled water for 5 seconds. The macrophage monolayer in each well was fixed with methanol and stained for microscopic examination.

The results of this experiment are average of two independent experiments, in each experiment, pooled PECs of two birds were used.

Table 2: Effect of Sephadex G-50 dose on the phagocytic activity of chicken peritoneal macrophages

Phagocytic cells (%)	Dose of 3% suspension of Sephadex G-50 (ml/100g b. wt.)	
	1*	2**
Non-opsonized SRBC	46.66 ± 13.2a	13.45 ± 3.6b
Opsonised SRBC	86.82 ± 3.1a	84.81 ± 5.7a

*It was injected on left side of the cavity. **Equal volume was injected on both sides of the cavity. The values in each row with different letters are significantly different (P<0.00: Student T test). The PECs were pooled from three birds and each figure is Mean ± SD of six observations.

Table 3: Effect of exposure time on phagocytic activity of chicken peritoneal macrophages

Time of phagocytic reaction (minutes)	Mean ± SD of Phagocytic cells	
	Non-opsonised (%)	Opsonised (%)
10	1.7 ± 1.2a (1)	40.2 ± 5.8a(4)
20	12.0 ± 4.9b(1)	72.6 ± 10.8b(8)
30	10.2 ± 1.0b(1)	85.7 ± 2.2c(9)

Each value represents the Mean ± SD of phagocytic cells of three independent experiments. In each experiment, the PEC were collected from one bird. The values in parenthesis indicate the number of SRBC/macrophage. The values in each column were analyzed using an ANOVA test and Duncan's Multiple Range Test.

The values not with different letters in a column are significantly (P<0.05) different from each other.

Table 4: Comparative efficacy of the macrophages to phagocytose *Salmonella gallinarum* and sheep red blood cells

Phagocytic particle (n = 3)	Phagocytic macrophages (%)	
	Non-opsonised	Opsonised
S. gallinarum	12.5 ± 6.8a	78.8 ± 15.7a
SRBC	13.9 ± 2.9a	82.8 ± 06.0a

The student T test showed that there is no significant difference in phagocytic activity of chicken peritoneal macrophages to *S. gallinarum* or SRBC. The washed *S. gallinarum* suspension 1000 x 10/mL was opsonised with 0.5 unit of agglutinating units of antibody at 4°C for overnight. The washed SRBC suspension was opsonised with 0.5 HA units of antibody at 37°C for 30 minutes.

The percentage of chicken phagocytic PEM in *in vitro* culture is also influenced by exposure time of phagocytic particles (Table 3). Peak percentage of phagocytic cells is acquired more quickly for the PEM exposed to non-opsonised SRBC than those exposed to opsonised SRBC (20 and 30 minutes, respectively). It may be attributed to the limited phagocytic activity of the former, hence achieving the target earlier than the latter.

The phagocytic ability of chicken PEM to endocytose SRBC is comparable to that of *S. gallinarum*. There was no difference in percentage of phagocytic cells to PEM exposed to either type of phagocytic particles opsonised with sub-agglutinating level of specific antibodies (Table 4).

Moreover, present study showed that the age of *in vitro* culture of chicken PEM did not has any effect on the ability ofmacrophage to phagocytose the opsonised SRBC (Table 5). These results are supported by Sabet *et al.* (1977) who observed constant level of phagocytosis to opsonised SRBC of chicken PEM over 5 weeks of *in vitro* culture.

Table 5: Effect of culture age on phagocytic activity of chicken peritoneal macrophages

Time post-cultivation (hours)	Phagocytic macrophage (%)	Distribution of macrophages on the basis of number of SRBC/macrophage (%)					
		1-2	3-4	5-6	7-8	9-10	>11
01	76.5	09.9	19.8	15.8	23.8	15.8	16.8
03	82.0	16.7	21.3	20.2	14.2	08.3	16.7
05	90.0	65.3	10.2	09.6	11.7	28.4	30.9
24	88.0	05.3	25.5	14.9	16.0	21.3	16.5

The Sephadex G-50 recruited peritoneal exudate cells (PEC) from three chickens were pooled and incubated for 30 minutes. The adherent cells were washed and medium was replaced with fresh RPMI-1640 and cells were left in incubator for different times before an assay. Five percent SRBC were sensitized with 0.5 HA units of anti-SRBC antibody at 37°C for 30 minutes. The same SRBC were used at all times of phagocytic assay.

In summary Sephadex G-50 recruited chicken PEM were phagocytic to opsonised as well as to non-opsonised SRBC or *S. gallinarum*. The phagocytosis depended on time of exposure as well as the intensity of the ligand molecules on the particles but was independent of the culture age of macrophages.

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