IN VITRO PHAGOCYTIC ACTIVITY AND BACTERICIDAL POTENTIAL OF BCG-ACTIVATED ALVEOLAR MACROPHAGES FROM RABBITS, GUINEA PIGS AND RATS

Talha Khalid, H. Afzal, M . J. Arshad and M. K. Shafiq
Department of Veterinary Microbiology,
University of Agriculture, Faisalabad-38040, Pakistan

ABSTRACT

BCG treatment of rabbits, guinea pigs and rats yielded alveolar exudate cells (AEC) which had higher viability and increased potential of adherence to glass coverslips. Moreover, these AECs consisted of higher number of macrophages when obtained from animals treated with BCG. Recovery of macrophages from AECs was higher in rabbits than guinea pigs and rats. Different laboratory animals exhibited pronounced phagocytic and bactericidal activity when stimulated with BCG and compared with their respective controls. Opsonization resulted in enhanced macrophage activity.

INTRODUCTION

Several types of macrophages are derived from blood monocytes and among them the alveolar macrophages, located in the alveolar air spaces (Hocking and Golde, 1979), play a major role in resistance to respiratory infections. This cell population is essential in lung clearance mechanism and supports several anti-infectious functions (Maxwell and Marcus, 1968). Furthermore, macrophage function and disease susceptibility are highly inter-related.

Macrophage biology and functions are influenced by genetics and species variation (Qureshi and Miller, 1991). This study was conducted to establish baseline profiles of various macrophage functions in three laboratory animals viz., rabbits, guinea pigs and rats.

MATERIALS AND METHODS

Animals

Young male rabbits, guinea pigs and rats of 4-6 weeks of age, locally bred and reared were used as the source of alveolar macrophages. Each species of the animals was randomly divided into two groups of five animals each i.e, A and B. Group A served as experimental (BCG activated) while group B was sham-treated. The animals were kept under standard hygienic conditions, fed and given water *ad libitum.*

Isolation of Alveolar Exudate Cells (AECs)

Alveolar macrophages were collected from rabbits, guinea pigs and rats using the previously described Bacillus-Calmette Guerin (BCG) activation protocol (Ahmad *et al.*, 1993) . Briefly, a single injection of BCG ($6x10^6$ colony forming units of living *Mycobacterium bovis*) at the dose rate of 1ml 20 g^{-1} body weight was injected intravenously into each animal of group A, while group B was kept as control. Each of the animal was surgically exposed after 48 hours of injection. Alveolar exudate cells (AECs) suspension were collected by lung washing of exsanguinated animals (Charley *et al.*, 1983) with 300 ml of sterile saline (0.89%) containing sterile heparin (0.5 IU/ml). The saline suspensions collected from each of the animal into plastic tubes were spun at 500 X g at 4^0 C for 10-15 minutes. Immediately after centrifugation, the supernatant was discarded to obtain alveolar exudate cells. Then these AECs were resuspended with an equal amount of RPMI 1640 (Sigma & Co. USA) growth medium supplemented with 5% heat inactivated bovine foetal calf serum and antibiotics (100 U/ml penicillin and 50 μg/ml streptomycin).

Cell viability Assay

The viability of AECs was tested both in BCG activated group and control group by a previously described trypan blue exclusion technique (Philips, 1977).

Substrate Adherence Potential

To determine substrate adherence potential, the lung washings from five animals were pooled. One ml of 1 x 10^6 AECs was then added to each of the two Petri dishes containing four round coverslips. After incubation at 30^0C for 1 hour in an incubator, the cover slips were washed, fixed and stained. The

number of adherent macrophages from four randomly selected fields were scored for each coverslip at 1,000 X (Ahmad *et al.*, 1993). The results were expressed as mean adherent cells per sample.

Percentage of Macrophages in AECs

The AECs were allowed to adhere on glass coverslips and 200 adherent cells per coverslip were classified by morphological criteria as round or slightly amorphous, refractile or granular cells (Lucas and Jamroz, 1961) to determine the percentage of macrophages in AECs.

Sheep Red Blood Cells (SRBCs) Phagocytosis Assay

The phagocytic activity of macrophages was determined using an *in vitro* opsonized and unopsonized SRBC phagocytosis assay previously described by Miller and Qureshi (1992).

Bactericidal Assay

The bacterial killing assay (opsonized and unopsonized) was performed as described for chicken macrophages (Qureshi and Miller, 1991). A highly pathogenic Congo Red positive (CR+) strain of *E.coli* (Akhtar *et al.*, 1991) was used in this assay.

Statistical Analysis

The statistical analysis was done on the data thus collected and the treatment means were separated by least significant difference (LSD) test. Data was analyzed by the analysis of variance (ANOVA) technique (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Bacillus-Calmette Guerin (BCG) is avirulent and a potent stimulator of macrophages. As a vehicle for antigens it is required to evoke long lasting cell mediated immunity due to its strong adjuvant properties. It is known that all adjuvants stimulate macrophages which are thought to be improving immunogenicity through an increase in the amount of antigen on their surface and the efficiency of its presentation to lymphocytes, by the provision of accessory signals to direct lymphocytes towards an immune response and by the secretion of soluble stimulatory factors (e.g, interleukin-I) which influence the proliferation of lymphocytes (Roitt, 1991).

In the present study recovery of live alveolar exudate cells was significantly higher in BCG treated animals than non-treated controls (Table 1). However, different species i.e, rabbits, guinea pigs and rats yielded similar number of live alveolar macrophages. These results are in line with the findings of Maxwell and Marcus (1968) and Sorrell *et al.*(1978), who reported a high viability of PECs of BCG immunized laboratory animals. The results of present study are partially in line with Sawyer *et al.* (1954) reported the mean percentage of viable cells 83% in a study conducted on rat peritoneal exudate cells. It may be due to the difference in the source of exudate cells. Present study was conducted on alveolar exudate cells.

The AECs harvested from BCG stimulated animals, were allowed to adhere on glass coverslips to determine the number of adherent cells per microscopic field. Alveolar exudate cells from rabbits and guinea pigs stimulated with BCG showed a significant higher adherence potential than respective controls (Table 1). Charley *et al.* (1983) also reported that adherence of swine alveolar macrophages increases with the activation of another adjuvant i.e, lypopolysacchride (LPS). However, effect of BCG treatment on AEC of rats was not significant.

The adherent cells after staining with May-Grunwald-Giemsa stain were classified by morphological criteria (Lucas and Jamroz, 1961) to determine the percentage of alveolar macrophages in adherent AECs. All the three animal species when treated with BCG showed a significantly higher percentage of macrophages in adherent AECs when compared with their respective controls (Table 1). Yield of macrophages was higher in rabbits then other two species, this could be attributed to genetic and species differences. Aslam (1994) has also reported variation in the percentage of macrophages recovery from different genetic strains of poultry. These results corroborate the findings of Arshed (1995) who reported that Sephadex G-50 stimulation increases the percentage of macrophages in avian respiratory phagocytes.

Macrophages from all animals showed a significantly higher phagocytic activity against opsonized sheep RBCs when compared with unopsonized group (Table 2). It may be due to the presence of plasma membrane receptors on macrophages for the Fc region of immunoglobulin which contribute to the recognition and binding of opsonized particle (Roitt, 1991). The present findings are inconsonance with Arshed *et al.* (1996) who also observed that opsonization of sheep RBCs resulted in enhanced phagocytic activity of macrophages. Truscott *et al.* (1990) reported that opsonization of *Pasteurella multocida* and *Candida albicans* with specific antibodies resulted in increased phagocytosis and intracellular killing by avian mononuclear phagocytic cells. All animals treated with BCG also showed a significantly

Table 1: Properties of alveolar exudate cells from rabbits, guinea pigs and rats

Treatment	Rabbits	Guinea Pigs	Rats
Viability			
BCG	67.33 ab (57.70-76.66)	73.67 b (60.37-85.15)	70.00 ab (68.45-73.00)
Control	62.00 cd (56.31-67.91)	57.00 d (53.34-61.00)	62.33 cd (58.83-68.90)
Adherence Potential			
BCG	81.67 ab (78.11-85.36)	86.33 a (82.93-89.45)	57.33 d (54.00-61.85)
Control	68.33c (62.93-75.01)	53.00 d (39.41-67.91)	50.67 d (41.98-58.41)
Macrophage yield			
BCG	90.33 a (83.29-97.07)	67.00 bc (61.09-73.87)	69.67 bc (63.73-76.08)
Control	62.00 d (58.98-65.86)	38.33 e (36.09-40.59)	37.33 e (39.65-46.11)

All these values are in Percentage. Values bearing different letters in each criteria differ significantly ($P<0.001$)

Table 2: Percentage phagocytic activity of BCG-activated macrophages harvested from rabbits, guinea pigs and rats

Treatment	Rabbits	Guinea Pigs	Rats
Opsonized			
BCG	60.00 c (57.88-62.76)	71.33 b (68.64-73.99)	64.33 bc (60.00-69.15)
Control	32.67 d (29.89-34.90)	142.67 e (38.75-47.00)	34.33 d (29.18-40.26)
Unopsonized			
BCG	29.00 a (28.00-30.20)	32.33 c (30.30-34.00)	31.27 a (28.12-33.26)
Control	18.11 bc (16.89-22.33)	21.94 b (18.33-24.67)	20.16 ab (17.00-22.12)

Figures bearing different letters in each criteria differ significantly ($P<0.001$)

Table 3: Percentage bactericidal (Killed within 15 minutes) activity of BCG-activated macrophages harvested from rabbits, guinea pigs and rats

Treatment	Rabbits	Guinea Pigs	Rats
Opsonized			
BCG	38.33 b (30.02-47.01)	48.67 a (42.60-54.62)	35.00 b (31.05-39.12)
Control	12.00 d (08.00-16.00)	15.33 d (10.01-20.99)	25.67 c (90.02-39.47)
Unopsonized			
BCG	22.66 c (16.65-27.60)	26.66 b (18.90-33.69)	18.33 c (30.84-22.76)
Control	5.66 e (04.17-07.15)	13.33 d (09.86-16.82)	12.00 d (08.49-14.51)

Figures bearing different letters in each criteria differ significantly ($P<0.001$)

higher phagocytic activity when compared with their respective controls.

Alveolar macrophages from all animals treated with BCG showed significantly higher bactericidal potential against *E.coli* as compared to their respective controls (Table 3). These findings support the results of Aslam (1994) and Ahmad *et al.* (1995) who reported a significant higher killing percentage of *E.coli* in treated birds as chompared to their respective controls. Results of the present study are also comparable with those of Ahmad *et al.* (1993) who reported a relatively significant difference in bacterial killing of opsonized *E.coli* by peritoneal macrophages within 15 minutes in all the three animal species inoculated with BCG and their respective controls.

The study suggests that there is an enhanced recruitment of alveolar macrophages following treatment with BCG which are of prime importance in lung clearance mechanisms and engulfment of dead cells or debris etc.

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