IN VITRO PHAGOCYTIC ACTIVITY AND BACTERICIDAL POTENTIAL OF BCG-ACTIVATED PERITONEAL MACROPHAGES FROM RABBITS, GUINEA PIGS AND RATS[1]

Muhammad Tauseef Ahmad, Hameed Afzal, Nadeem Iqbal Chaudhry & M. Siddique
Department of Veterinary Microbiology, University of Agriculture, Faisalabad

ABSTRACT

The present study was conducted to establish baseline profiles of various macrophage functions in three commonly used laboratory animals viz., rabbits, guinea pigs and rats. All experiments were carried out between 4 to 6 weeks of age. Total number of peritoneal exudate cells per animal collected after a single intraperitoneal injection of Bacillus-Calmette Guerin (BCG) were comparable among all these three animal species. Rabbits produced fewer macrophages along with reduced phagocytosis of opsonised and unopsonised sheep red blood cells (SRBC). Macrophages from guinea pigs and rats killed more opsonised *Escherichia coli* than rabbits. This study demonstrates species variation among the three laboratory animals for mononuclear phagocytic system functions.

INTRODUCTION

The macrophages are derived from bone marrow promonocytes which, after differentiation to blood monocytes, finally settle in the tissues as mature macrophages where they constitute the mononuclear phagocyte system (Roitt, 1988). Disease resistance has at least two important aspects, i.e., it is under both humoral (antibody) and cell-mediated control.

Macrophages from H_1 and L_1 mice have been shown to phagocytose C3 (IgM)-coated sheep red blood cells (SRBC) after infection with Pasteur strain of rabies virus (Consales *et al.*, 1990). Enhanced internalisation of opsonised SRBC by prolonged enhancement of macrophage activity during *Brucella* infection (Birmingham & Jeska, 1991), ochratoxin A (Boorman *et al.*, 1984) and *Plasmodium berghei* (Abdel-Hafez *et al.*, 1986).

Macrophages, in addition are known to play a multifaceted role such as tumour cell killing (Hamilton & Adams, 1987) and several biologically active monokines such as interleukin-1 and tumour necrosis factor (Kovacs *et al.*, 1988). It is also clear that macrophage function and disease susceptibility are highly interrelated. Evidence from both mammalian system indicates that various macrophage functions are influenced greatly by species differences.

Based on the knowledge of macrophage biology and functions, as well as the fact that such functions are influenced by species differences, this challenge study was conducted to establish baseline profiles of various macrophage functions in the three commonly used laboratory animals viz., rab-

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bits, guinea pigs and rats. This study may also be fruitful in developing strategies for improving the disease resistance potential of laboratory animals, poultry stocks, livestock and above all, the man.

MATERIALS AND METHODS

Young male guinea pigs, rabbits and rats of 4-6 weeks of age were used as the source of peritoneal macrophages. Each species of the animals was randomly divided into two groups A and B. Group A served as experimental while group B was sham-treated. The animals were kept under standard hygienic conditions, fed commercial chow and given water for *ad libitum* access.

Isolation of Peritoneal Exudate Cells (PEC): Macrophage-rich PEC were collected from guinea pigs, rabbits and rats using previously described Bacillus-Calmette Guerin (BCG)-activation (Johnson *et al.*, 1981) protocol.

Briefly, a single injection of each of BCG (6×10^6 colony-forming units of living *Mycobacterium bovis*) (Meltzer & Oppenheim, 1977) was injected intraperitoneally into each animal of group A. Group B was sham-treated. The animals were euthanised after 48 hours of intraperitoneal injection. From 20-30 mL of sterile saline (0.89%) containing sterile heparin (0.5 IU mL^{-1}) was injected rapidly into the peritoneal cavity and the fluid was circulated by prodding the anterior abdominal wall. The saline suspension collected from each of the animal into plastic tubes were spun at 1500 rpm for 10-15 minutes in a refrigerated centrifuge. Immediately after centrifugation, the supernatant was discarded to obtain PEC.

Macrophage Cultures: PEC from individual animals were resuspended in RPMI 1640 growth medium supplemented with 5% heat-inactivated bovine fetal calf serum and antibiotics (100 U mL^{-1} penicillin and 50 μg mL^{-1} streptomycin).

To determine the incidence of macrophages, total non-erythroid PEC from each of the animal were counted on a haemacytometer. One mL PEC suspension from each of the five animals per group was transferred into petridishes containing four sterile round coverslips. Petridishes were incubated at 37°C for one hour to allow macrophages' adherence. Coverslips were then washed with sterile saline (0.89%) to remove all non-adherent cells and other debris, fixed in methanol, stained with May-Grunwald-Geimsa, mounted on clean glass slides, and approximately 200 adherent cells per coverslip were determined morphologically under microscope at 1,000 x.

Sheep Red Blood Cell (SRBC) Phagocytosis Assay: The phagocytic activity of macrophages belonging to different animal species was determined using an *in vitro* SRBC phagocytosis assay (Qureshi *et al.*, 1986; Pereira *et al.*, 1987).

Freshly collected SRBC were washed thrice in sterile PBS and a 5 per cent suspension coated with a subagglutinating concentration of specific hyperimmune antiserum (heat inactivated at 56°C for 30 minutes). The SRBC, after washing with PBS, were resuspended in RPMI 1640 growth medium. A 5 per cent suspension of unopsonised SRBC separately prepared in RPMI 1640 growth medium was kept as control.

Pooled PEC suspensions from five animals per species were used to establish macrophage monolayers for the phagocytosis assay. The viable macrophage concentration was adjusted to 1×10^6 mL^{-1} by the trypan blue exclusion technique (Philips, 1977). The adherent PEC monolayer rich in macrophages, was prepared in triplicate dishes on round coverslips. The culture medium from each set of three petridishes

was removed and 1.0 mL of 5 per cent opsonised and unopsonised SRBC suspension was dispensed into each petridish. The cultures were incubated at 37°C for 60 minutes. The coverslips were then washed with saline, fixed in methanol and stained as before. Index of ingestion which is the percentage of cells with ingested SRBC multiplied by the average number of SRBC ingested per cell was calculated. At least, 200 cells were scored for the percentage of ingestion and for the average number of SRBC ingested (Consales *et al.*, 1990).

Bactericidal Assay: The bacterial killing assay was performed as described for bovine (Desiderio & Campbell, 1983) and chicken (Qureshi *et al.*, 1986) macrophages. A logarithimic-phase culture for 30 minutes at 37°C with a subagglutinating concentration of specific antiserum was taken. Opsonised and unopsonised *E. coli* were fed to macrophage monolayers at a ratio of approximately 25:1 bacteria to macrophage. After 15 minutes of incubation, coverslips were removed, washed in saline containing 1 per cent bovine fetal calf serum, and transferred to culture dishes containing fresh medium. The number of viable intracellular bacteria at each point was expressed as a percentage of the number initially interiorised by the macrophages.

The data thus collected were subjected to statistical analysis and the treatment means were separated by Least Significant Difference (LSD) test (Steel & Torrie, 1980).

RESULTS AND DISCUSSION

Incidence of adherent macrophages from rabbits, guinea pigs and rats elicited with BCG and in sham-treated animals, PEC onto glass coverslips was determined. The percentage of adherent macrophages in PEC of rabbits, guinea pigs and rats in response to BCG was low in rabbits and statistically non-significant in guinea pigs and rats (Table 1).

Table 1. Incidence of macrophages in BCG-activated PEC in rabbits, guinea pigs and rats

Treatment	Macrophages (%)		
	Rabbits	Guinea pigs	Rats
BCG	67.51 bc	91.27 a	85.18 a
Sham	32.56 e	50.79 d	39.64 e

Figures sharing at least a letter in common are statistically non-significant ($P \leq 0.05$).

Mediator activated macrophages exhibited increased stickiness and ruffled membrane motility and it is of note that increased macrophage adherence is a biologic effect of macrophage migration inhibition factor (MIF) (Piessens *et al.*, 1975). These results are in line with those of Dailey *et al.* (1977) and Sorrell *et al.* (1978) who reported a 61 per cent macrophage yield in *Corynebacterium parvum*-elicited C3H/HJ mice and 75 to 90 per cent macrophages in guinea pigs injected intraperitoneally with mineral oil, respectively. The difference in macrophage yield appears to be related to the affinity characteristics of the various receptor site interactions involved in the expression of mediator activity.

When macrophage monolayers from rabbits, guinea pigs and rats inoculated intraperitoneally with BCG were incubated with opsonised and unopsonised sheep red blood cells (SRBC), statistically significant differences ($P \leq 0.05$) were observed between rabbits and guinea pigs and, rabbits and rats but difference between guinea pigs and rats was non-significant. However, in sham-

treatment, there was a significant difference between rabbits and guinea pigs and, guinea pigs and rats but this was non-significant between rabbits and rats. Macrophages from guinea pigs and rats exposed to BCG exhibited enhancement in phagocytic potential for opsonised and unopsonised SRBC that was statistically significant ($P \leq 0.05$) from thioglycollate broth-induced and sham-treated animals (Table 2).

Table 2. Phagocytic potential of BCG-activated macrophages from rabbits, guinea pigs and rats

Treatment	Phagocytic macrophages (%)		
	Rabbits	Guinea pigs	Rats
	..... Opsonised		
BCG	43.56 g	83.35 a	80.89 a
Sham	35.24 hi	52.27 ef	35.09 hi
	..... Unopsonised		
BCG	34.84 hi	70.62 bc	71.27 b
Sham	28.93 i	42.01 gh	31.11 i

Figures sharing at least a letter in common are statistically non-significant ($P \leq 0.05$).

The depression in phagocytic activity in sham-treated animals may be related to inhibition of molecular synthesis in macrophages after exposure to BCG, or alterations in metabolic processes principally glycolysis essential for phagocytosis, and/or an alteration in the macrophage membrane as reported by Qureshi *et al.* (1989).

The mechanism by which BCG-sensitised macrophages from rabbits suppressed the phagocytic response of opsonised and unopsonised SRBC may be due to soluble suppressive factors released by cultured macrophages. This may perhaps be also due to different states of activation (Weiss & Fitch *et al.*, 1978). The results are in agreement with the findings of Consales *et al.* (1990) who studied the intrinsic activity of macrophages from H_1 and L_1 mice and showed that phagocytosis of C3 (IgM) SRBC ranged 21 to 80 and 75 to 96 per cent in H_1 and L_1 mice infected with Pasteur strain of rabies virus. These findings also extend a number of previous studies of Birmingham and Jeska (1981), Boorman *et al.* (1984) and Abdel-Hafez *et al.* (1986).

The results of the effect of BCG-activation and sham-treatment showed a non-significant difference in bacterial clearance within 15 minutes in rabbits and rats (against opsonised and unopsonised *E. coli*). However, the difference was also non-significant in BCG and sham-treated guinea pigs against opsonised *E. coli*. Both opsonised and unopsonised *E. coli* were killed quite efficiently by peritoneal macrophages from guinea pigs and rats compared with rabbits when these were elicited with BCG. However, macrophages from sham-treated guinea pigs were more efficient than those of rabbits and rats in their bactericidal ability (Table 3).

Table 3. Bactericidal potential of BCG-activated macrophages from rabbits, guinea pigs and rats

Treatment	Per cent bacteria killed within 15 minutes		
	Rabbits	Guinea pigs	Rats
	..... Opsonised		
BCG	24.00 cde	36.00 a	34.66 ab
Sham	16.00 efg	28.00 abcd	16.00 efg
	..... Unopsonised		
BCG	29.33 abcd	33.33 abc	30.66 abcd
Sham	12.00 fgh	28.00 abcd	12.00 fgh

Figures sharing at least a letter in common are statistically non-significant ($P \leq 0.05$).

The difference in their ability to mount a variable bactericidal potential in all the three animal species in response to BCG and sham may be due to the fact that both guinea pig's and murine macrophages are activated, directly or indirectly, produce lysosomal enzyme secretion, collagenase and lysozyme (muramidase). Furthermore, macrophage activation is in fact a heterogeneous phenomenon and that the expression of activity by the cell is dependent on the particular mechanism of activation (Wilton *et al.*, 1975).

In conclusion, BCG may be a batter activating agent for recruitment of PEC and guinea pigs for maximum yield of macrophages. This study demonstrates functional variation between three laboratory animals for mononuclear phagocyte system which may now be used more efficiently as experimental model for immunological procedures. This system may also be manipulated for evaluation of vaccines and antibiotics which will be fruitful in the field of modern immunology.

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