

ACQUIRED RESISTANCE OF MICE AGAINST *S. MANSONI*  
AND LUNG GRANULOMATOUS REACTION INDUCED BY BCG

M. TENDLER\*  
R. MAGALHÃES PINTO\*  
E.A. BAMBIRRA\*\*  
M. QUEIROZ CRUZ\*\*\*  
A. OLIVEIRA LIMA\*\*\*\*

*Studies carried out in Sw outbred mice showed that there is no correlation between the degree of lung granulomatous reaction and the level of acquired resistance against S. mansoni infection induced by BCG.*

Treatment of mice with BCG has been shown to produce a marked resistance against a variety of infections and tumors (Old et al, 1961; Capron & Lesoin, 1969; Ortiz-Ortiz, Gonzalez-Mendoza & Lamoyi, 1975). The resistance against *S. mansoni* infection induced by BCG has been the subject of many studies (Fauve & Dodin, 1976; Bout et al, 1977 & Civil & Mahmoud, 1977). This resistance is dependent on mouse strain, dose, route and time of administration of the mycobacteria and appears to be mediated through activated macrophages (Civil et al, 1978; Maddison et al, 1978).

The studies reported in this paper were undertaken to determine whether the protective effect of BCG against *S. mansoni* infection require granuloma formation or whether this protection is closely related to the extension of lung granulomatous reaction presented by the animals.

#### MATERIAL AND METHODS

*Mice:* Male Sw random bred mice, weighting 18-22g, were obtained from Instituto Oswaldo Cruz.

*BCG:* Lyophilized preparation of BCG (BCG Moreau-Rio de Janeiro) containing  $5 \times 10^7$  CFU per ampoule was obtained from Fundação Ataulpho de Paiva, RJ. The

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\*Instituto Oswaldo Cruz, CNPq Research Fellow, Caixa Postal 926 – 20000, Rio de Janeiro, Brazil.

\*\*Universidade Federal de Minas Gerais, Faculdade de Medicina, Deptº de Anatomia Patológica, Minas Gerais, Brazil.

\*\*\*Instituto de Puericultura e Pediatria Martagão Gesteira, Rio de Janeiro, Brazil.

\*\*\*\*Centro de Pesquisa Arlindo de Assis, Rio de Janeiro, Brazil.

bacilli were reconstituted to 5ml with sterile pyrogen-free 0.85% NaCl solution to contain  $1 \times 10^7$  CFU/ml. Heat-killed BCG was prepared by exposure of the reconstituted BCG to 65°C for 90 min.

*Vaccination with BCG:* Four schedules for i.v. vaccination were used. In the first two groups, each mouse received  $10^7$  viable or dead BCG bacilli in 0.2ml of an oil-in-water emulsion containing 10% mineral oil and 0.2% Tween 80; in the other two groups each mouse received  $10^7$  viable or dead BCG bacilli in saline. Control animals received only saline, without BCG. For each experiment, 10 mice per group were used.

*Challenge with S. mansoni:* BCG-treated and control mice received subcutaneously 100 cercariae of *S. mansoni*, L.E. strain. This challenge was done 10 days after immunization with BCG in saline and 30 days after BCG emulsified in oil, so that different degree of lung granulomatosis could be compared.

*Assay of parasite infection:* Parasite load was determined either by schistosomula recovered from lungs 6 days after exposure to cercariae, or by perfusion of adult worms from the portal and mesenteric veins, 45 days after challenge. The percentage of protection was calculated as follows:

$$\% \text{ protection} = \frac{\text{parasites recovered from control mice} - \text{parasites recovered from BCG-treated mice}}{\text{parasites recovered from control mice}} \times 100$$

*Histopathology:* At various periods of time after i.v. administration of BCG, mice were sacrificed and their lungs removed for histologic examination. Tissues were fixed in 10% phosphate-buffered formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin.

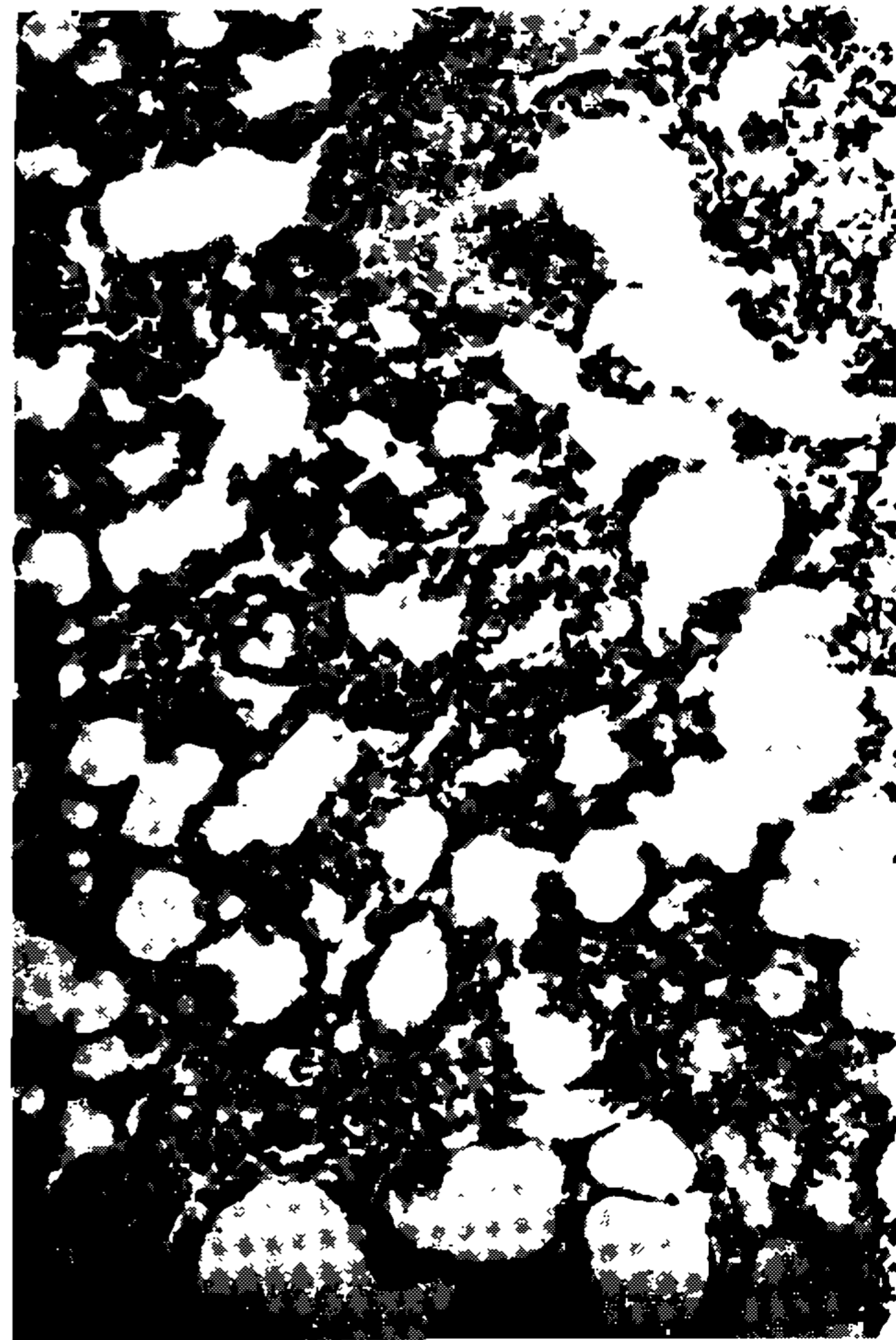
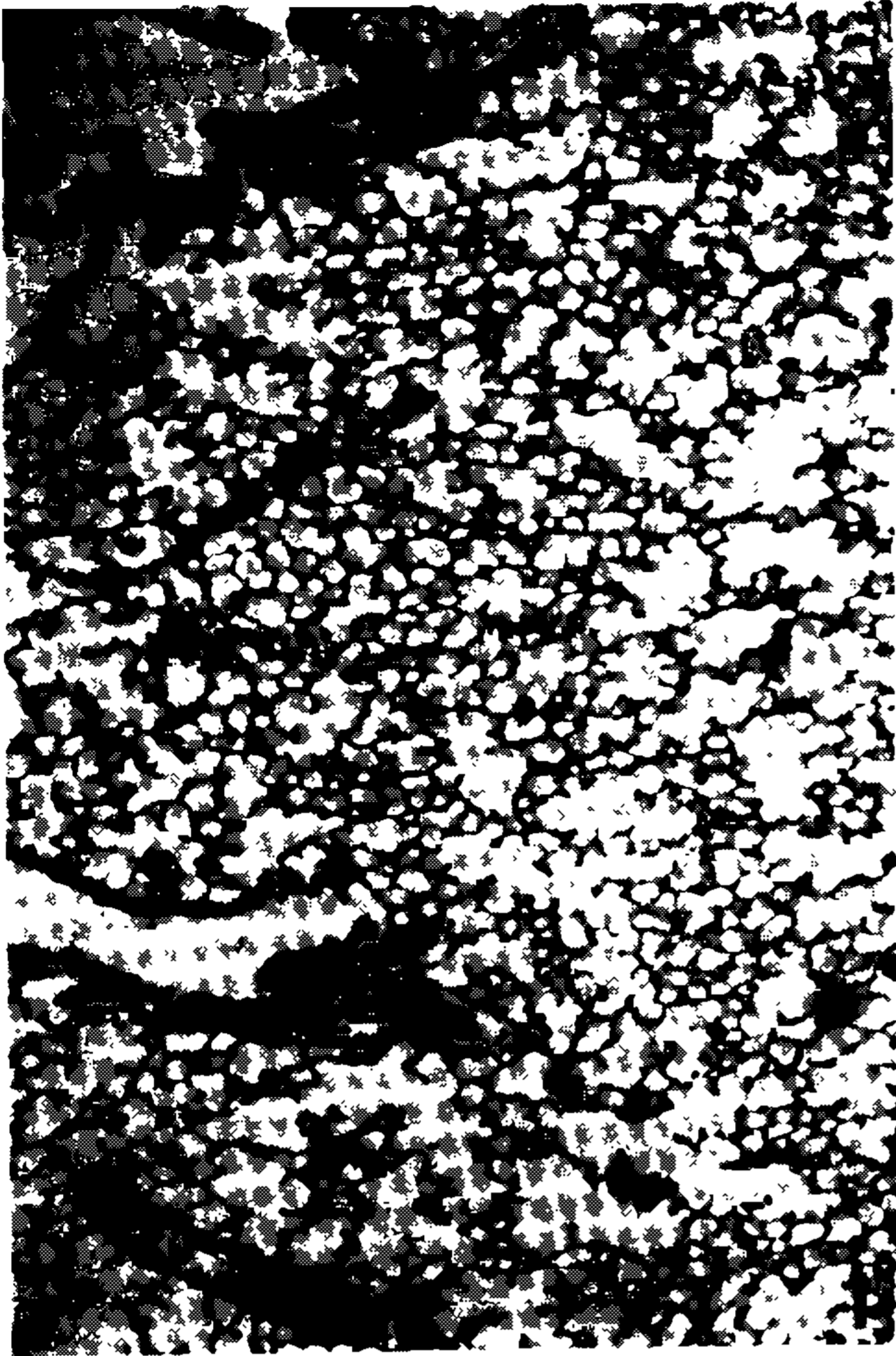
## RESULTS

The results obtained showed that the protection induced by i.v. vaccination of mice with BCG reached the level of 70% using viable bacilli in saline and only 19% with dead bacilli in saline in identical experimental conditions. The protection induced by the same dose of BCG in oil-water emulsion reached the level of 13% with viable and of 24% with dead bacilli (Table I). Similar results were obtained either by schistosomula lung recovery or by perfusion of adult worms from the liver.

The histologic sections of lungs from animals that received BCG in saline (viable or dead bacilli) showed a well preserved structure with rare and sparse granulomas and slight septal thickness infiltrated by mononuclear cells (Figs. 1 and 2). Mice vaccinated with BCG in oil showed an intense septal thickness produced by mononuclear cell infiltration, and numerous granulomas scattered throughout the pulmonar parenchyma (Fig. 3 and 4).

## DISCUSSION

The intravenous administration of  $1 \times 10^7$  viable BCG bacilli in saline to Sw outbred mice conferred a high degree of nonspecific protection (70%) against *S. mansoni* infection. The same amount of viable BCG in an oil-in-water emulsion injected i.v. only produced a low level of protection (13%). A similar high degree of resistance (70% with BCG in saline) has also been produced with other nonspecific biologic agents such as *E. coli* (Smith et al, 1975), *T. gondii* (Mahmoud, Warren and Strickland, 1976), *C. parvum* (Mahmoud, et al, 1979) and cord factor (Mahmoud, et al, 1977). The mechanism whe-



Figs. 1 and 2 – Lung at 7 days after intravenous injection of BCG in saline. Preservation of the alveolar septal structure with mild mononuclear cell infiltration (hematoxylin & eosin, original magnification: 1. X120; 2. X300).

Figs. 3 and 4 – Lung from mouse injected with BCG in oil. At 7 days after the injection, extensive septal infiltrate determining intense septal thickness is composed of mononuclear cells and sparse microgranulomas around oil droplets. (hematoxylin & eosin, original magnification: 3. X120, 4. X300).

reby BCG suppresses a schistosome infection is not yet completely elucidated. This resistance has been reported to be dependent on mouse strain, dose, route and time of administration of the mycobacteria (Maddison, Chandler & Kagan, 1978). It develops within a few days, cannot be transferred with serum or cells, is not mediated by eosinophils, does not require phagocytosis and appears to be due to substances elaborated by activated macrophages (Civil & Mahmoud, 1978; Maddison, Chandler & Kagan, 1978; Olds et al, 1980).

To clarify further the influence of macrophages on the mechanism of resistance induced by BCG in Sw mice against *S. mansoni* infection several experiments were carried out by us to assay the resistance of mice having the lungs previously infiltrated with large number of macrophages. Using i.v. injections of BCG in saline and in an oil-water emulsion it was possible to induce the desired lung infiltration with different degrees of granuloma formation. Emulsions with the largest oil droplets were found to be the most granulomatogenic. It was reasoned that during their migration through the lungs the parasites would encounter on their way to portal veins an adverse cellular reaction that would be proportional to the number of macrophages or granulomas present in the lungs. As we can see (Table I) this assumption was not confirmed. There was no direct correlation between the degree of resistance and the intensity of granulomatous tissue present in the lung. Actually the degree of protection was greater in mice that received BCG in saline and with lower numbers of lung granulomas.

TABLE I

Protection against *S. mansoni* infection induced in SW outbred mice by intravenous injection of BCG. Viable and dead bacilli injected in saline or emulsified in oil-in-water mixture.

<i>BCG in oil-in-water</i> (i.v.)	(% Protection)	
	<i>Viable bacilli</i>	<i>Dead bacilli</i>
	13	24
<i>BCG in saline</i> (i.v.)	70	19

Such a relationship between acquired resistance, portal hypertension and lung granulomas has been described in responder strain of mice infected with *S. mansoni* (Dean, Bukowski, Cheever, 1981) but our experiments apparently offer no support to the contention that schistosomula might be nonspecifically killed in the lungs by macrophages. Nevertheless, it would be interesting to investigate further the cytotoxic activity of the macrophages obtained from those lung granulomas.

## RESUMO

Pesquisas realizadas em camundongos Sw, não singeneicos, mostraram não ter havido correlação entre a intensidade da reação granulomatosa pulmonar e o grau de resistência adquirida contra a infecção por *S. mansoni* induzida pelo BCG.

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