Rev. Inst. Med. trop. São Paulo
38 (5): 359-363, setembro/outubro, 1996

EFFECTS OF NON-SPECIFIC IMMUNOPOTENTIATORS IN EXPERIMENTAL *Schistosoma mansoni* INFECTION. II. *Corynebacterium parvum.*

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SUMMARY

The effects of *Corynebacterium parvum* on host protection, tissue reaction and "in vivo" chemotaxis in *Schistosoma mansoni* infected mice were studied. The *C. parvum* was given intraperitoneally using a dose of 0.7 mg, twice a week (for 4 weeks), thirty days before (prophylactic treatment) or after infection (curative treatment). The host protection was evaluated through the recovery of adult worms by liver perfusion and was lower in the prophylactic group as compared to the control group ($p = 0.018$), resulting in 44% protection. The "in vivo" leukocyte response in both prophylactic and curative groups was higher as compared to the infected/non treated group ($p = 0.009$ and $p = 0.003$, respectively). Tissue reactions were described in the experimental and control groups, but there were not remarkable differences among them. The possible biological implications and relevance of the findings for the defensive response of the host and control of schistosomiasis are discussed.

KEYWORDS: *Corynebacterium parvum*; *Schistosoma mansoni*; Immunopotentiators; Chemotaxis.

INTRODUCTION

The immune response can be non-specifically stimulated by a number of agents[17,25]. One of them, *C. parvum*, has been extensively studied in rodents, and its multiple effects are known, such as: 1) antitumour activity; 2) increased bone marrow monocyte production; 3) mononuclear phagocytic system (MPS) stimulation; 4) macrophage activation; 5) enhanced antibody response and 6) increased resistance to bacterial and viral infections[11,18].

C. parvum can induce stimulation or suppression of the immune system depending upon the route of infection[10,29,30]. When administered by the intraperitoneal route, it causes high antibody titres, peaking after one month. When the subcutaneous route is used, *C. parvum* causes little or no antibody response[31]. This agent has been used in several biological systems, leading to variable results[2,15]. However, little is known about its action in schistosomiasis. In addition, there are many studies suggesting that the immune response to *S. mansoni* can be affected by non-specific mechanisms[5,21]. For these reasons, we decided to investigate whether *C. parvum* could enhance non-specific resistance against *S. mansoni* in an experimental model. The study was particularly focused on evaluation of protection of the host, granulomatous tissue reaction and "in vivo"chemotaxis.

MATERIALS AND METHODS

Animals and infection. Six to eight week old C57B1/10 male mice were obtained from the animal facilities of CPqAM-FIOCRUZ, Recife. Experimental and

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control groups were infected percutaneously with 90 cercariae (BH strain)[14] shed from laboratory infected *Biomphalaria glabrata*.

Drug adminstration. The *C. parvum* was supplied by Institute of Antibiotics/ Universidade Federal de Pernambuco. It was used intraperitoneally, at a dose of 0.7 mg/day, twice a week (during 4 weeks), thirty days before (prophylactic treatment) or after infection (curative treatment).

Experimental groups. Three experimental groups (ten mice each) were used to investigate three different issues: immune protection, tissue reaction and "in vivo" leukocyte chemotaxis. The effects of *C. parvum* were evaluated both prophylactically and curatively. The experimental groups were compared with their respective controls (non-treated infected mice). In the particular case of "in vivo" chemotaxis, an additional group of non-infected and non-treated mice was also used.

Animals experiments have been performed as to assure a minimal suffering for the animals.

"In vivo" **Leukocyte chemotaxis**. Ten mice were subjected to experiments using the air pouch technique of LAWMANN et al.[13] as modified by ABATH et al.[1] 50 days after infection by *S. mansoni*. One tenth millilitre of a soluction of Staphylococcal A protein (100 ng/ml) was injected into an air pouch produced subdermally on the back of mice. Two hours later, the connective tissue air pouch was excised and the thin membranous lining of the pouch was microscopically examined after Giemsa staining. The number of leukocytes in 10 randomly selected microscopic fields (x 400) were scored.

Assessment of protection. The degree of protection to a live infection, elicited by *C. parvum* treatment, was measured by infecting the mice and perfusing the liver 60 days later according to SMITHERS & TERRY[26]. The number of worms recovered from each treated group of mice was compared with a non-treated control group, and protection was expressed as a percentage according to the following formula:

$$\text{Protection} = \frac{C\text{-}E}{C} \times 100, \text{ where:}$$

C - mean number of worms from the control group;
E - mean number of worms from the treated group.

Histopathological studies. Ten mice were killed on the 60 th day of infection and samples from the liver and intestines were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 5 µm and stained with haematoxylin-eosin.

The hepatic granulomas were counted and measured by an ocular micrometer (x10) that was calibrated through a stage micrometer. Only newly formed granulomatous lesions were measured, e.g. granulomas containing an eosinophilic miracidium.

Statistical analysis. Statistical comparisons were made by analysis of variance, with a level of significance for **p** set at 0.05.

RESULTS

In Table 1, the polymorphonuclear (PMN) leukocyte response, in the infected and non-treated groups was significantly lower than in the non-infected and non-treated one. In order to evaluate if the defective response could be reversed, two kinds of *Corynebacterium parvum* treatments were used. Both prophylactic and curative groups showed a significant increase in chemotaxis as compared to the infected and non-treated group ($p = 0.009$ and $p = 0.003$, respectively). When the prophylactic and the curative groups were compared, the former showed a stronger PMN leukocyte response. However,

TABLE 1
Leukocyte response in *S. mansoni* infected mice treated prophylactically and curatively by *C. parvum* and controls

Groups	Leukocyte response[a]	
	PMN	MN
Non-infected/non treated	193.85 ± 80.11*	2.78 ± 2.16
Infected/non treated	11.98 ± 7.83	2.36 ± 1.25
Prophylactic	125.47 ± 122.57*	4.87 ± 3.92
Curative	71.47 ± 54.73*	2.01 ± 0.70

Each group was composed of ten mice.
[a] Means ± standard deviations;
PMN, polymorphonuclear; MN, mononuclear
* Statistically significant in comparison to the infected/non treated group.

TABLE 2
Ability of the prophylactic and curative *C. parvum* treatments to induce protection in mice.

Groups	Adult worm recovery[a]	Protection %
Control (infected / non treated)	23.4 ± 8.82	
Prophylactic	13.2 ± 6.64*	44
Curative	18.5 ± 6.02	21

Each group was composed of ten mice
[a] Means ± standard deviations.
* Statistically significant in comparison to the control.

the levels, of PMN leukocyte response never reached the levels observed in non-infected and non-treated mice. The prophylactic *C. parvum* treatment seemed to be more efficient than the curative treatment as far as recovery of normal chemotaxis is concerned.

The total number of adult worms recovered was significantly lower in the prophylactic group as compared to the control group ($p = 0.0018$), resulting in 44% protection (Table 2). The number of adult worms recovered from the curative group, although lower than in the last group, did not result in statistical significance. As expected, the number of granulomas was also lower in these groups.

Histopathologically, some liver granulomas seemed to be more exuberant in the group subjected to the curative treatment and in the infected non-treated mice, as compared to the prophylactic group. In this group an early and extensive collagen deposition was observed in periovular granulomas. However, differences concerning mean diameter of granulomas were not significant when prophylactic, curative and control groups were compared. The number of granulomas in the prophylactic, but not the curative group was lower than in the control ($p = 0.002$, Table 3). In the intestines, single eggs and periovular granulomas were more numerous in the walls of the jejunum-ileum. They were present in all the histological layers in both experimental and control groups, were predominantly of the exsudative type and seemed to be of a smaller size as compared to those seen in the liver.

TABLE 3
Measurements and number of granulomas in experimental and control groups

Groups	Granuloma[a] Number	Mean Diameter (mm)
Control (infected/non treated)	25.5 ± 9.63	0.38 ± 0.05
Prophylactic	13.7 ± 4.24*	0.35 ± 0.02
Curative	21.3 ± 7.04	0.35 ± 0.02

The granulomas were counted and measured in liver sections of the same size, corresponding to ten mice.
[a] Means ± standard deviations
* Statistically significant in comparison to the control.

DISCUSSION

The present investigation corroborates previous studies showing that *S. mansoni* infections cause a defective chemotactic response due to a decrease in the production and mobilization of PMN[24,27]. A chemotaxis defect was also demonstrated in other parasitic diseases, such as Chagas's disease[3]. The biological role of the defect in chemotactic response found in experimental schistosomiasis is not clear at the moment. The prophylactic *C. parvum* treatment seemed to be more efficient than the curative treatment as far as recovery of chemotaxis is concerned. In addition, the number of adults worms recovered was significantly lower in the prophylactic group than in the control, resulting in 44% protection. Thus, chemotaxis seems to be one of the various components of the inflammatory response stimulated by *C. parvum* that can be important for killing the parasite.

The degree of protection was evaluated through the recovery of adult worms by perfusion of the portal mesenteric system. It is important to mention that no adhesion of tissues and organs in the mouse abdomen was observed, that could interfere with the efficacy of the portal perfusion technique used (this may happen with some substances injected intraperitoneally). The 44% protection reported in the present paper is comparable to the immunity conferred by some schistosomiasis vaccine candidates[6]. The reason why the prophylactic treatment leaded to protection could be explained by the fact that the young schistosomula (the invasive stage of the parasite) are more susceptible to host effector defensive mechanisms than older worms. The *C. parvum* can activate macrophages (and consequently produce complement chemotactic fragments) and stimulate B cells[4,7,15,16]. It has been used in anticancer therapy as well as in several infectious diseases[2,4,19]. Recently, it was shown that macrophages become activated to produce toxic nitric oxide and to kill shistosomula after "in vitro" stimulation by combinations of the cytokines IFN-γ, TNF-α, IL-1 and IL-2, and this phenomenon is important for protective immunity to schistosomiasis[12]. There is evidence that these effector mechanisms may also operate "in vivo"[28].

The effects of levamisole, another immunopotentiator, on experimental schistosomiasis were different from *C. parvum*[21]. It was previously shown that levamisole seems to increase the susceptibility of inbred C57B1/10 mice to the infection. The net effect of non-specific immunopotentiators depends on several factors: host immune status, severity of infection dose and timing of drug administration[8,9,22,23] and, of course, the specific action of each of them.

The present data suggest that the *C. parvum* treatment before the *S. mansoni* infection is more efficient in protecting against infection, probably due to the following reasons: a) macrophage activation before infection; b) greater efficiency in increasing the leukocyte response; c) greater susceptibility of schistosomula. Thus, macrophage activation and leukocyte chemotaxis

(phenomena which can occur in the abscence of immunological control and are part of the inflammatory reaction) seem to be potentially relevant for the protective response in mice. In this regard, since cell adherence reactions and ensuing surface damage are rarely identified in schistosomula that have accomplished migration out of the epidermis, the initial and crucial event in protective immunity may well be an inhibition, by an inflammatory reaction, of development of migration of the parasites rather than by direct immune killing. Once the parasite is immobilized, its ability to continue circumventing immune attack may then be overcome[20].

In parallel with the development of vaccines against schistosomiasis, the search for more efficient adjuvant systems is being carried out[6]. In this regard it is encouraging that *C. parvum* could induce a degree of protection comparable to some *S. mansoni* vaccine candidates. Based on the present findings it would be relevant to investigate this immunopotentiator in association with vaccine candidates, specific chemotherapeutics and in other administration schedules.

RESUMO

Efeitos de imunopotenciadores não específicos na infecção experimental pelo *Schistosoma mansoni*. II. *Corynebacterium parvum*.

Neste trabalho foram avaliados os efeitos do *Corynebacterium parvum* na proteção do hospedeiro, reação tecidual e quimiotaxia "in vivo" em camundongos infectados pelo *S. mansoni*. O *C. parvum* foi dado intraperitonealmente usando uma dose de 0,7 mg, duas vezes por semana (durante 04 semanas), 30 dias antes (tratamento profilático) e 30 dias após a infecção (tratamento curativo).

A proteção do hospedeiro foi avaliada através da contagem de vermes adultos obtidos através da perfusão hepática de camundongos infectados e esse número foi bem menor no grupo profilático comparado ao grupo controle ($p = 0,018$), obtendo-se 44% de proteção. A resposta quimiotática "in vivo", nos grupos curativo e profilático, foi maior do que no grupo infectado/não tratado ($p = 0,009$ e $p = 0,003$, respectivamente). As reações teciduais foram descritas em todos os grupos, embora não tenha ocorrido diferenças marcantes entre eles.

As possíveis implicações biológicas e a relevância dos achados para a resposta defensiva do hospedeiro e controle da esquistossomose são discutidas neste trabalho.

ACKNOWLEDGEMENTS

We are grateful do Dr. Tetsuo Tashiro for statistical assistance and Dr. Clécio Santana for providing the *Corynebacterium parvum*.

This investigation received financial support from CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and FACEPE (Fundação de Amparo à Ciência e Tecnologia).

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Recebido para publicação em 27/02/1996
Aceito para publicação em 17/10/1996