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/untreated 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. One of them, C. parvum, has been extensively studied in rodents, and its multiple effects are known, such as: 1) antitumor activity; 2) increased bone marrow myelocyte production; 3) mononuclear phagocytic system (MPS) stimulation; 4) macrophage activation; 5) enhanced antibody response and 6) increased resistance to bacterial and viral infections.

C. parvum can induce stimulation or suppression of the immune system depending upon the route of infection. When administered by the intraperitoneal route, it causes high antibody titers, peaking after one month. When the subcutaneous route is used, C. parvum causes little or no antibody response. This agent has been used in several biological systems, leading to variable results. 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. 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 C57Bl/10 male mice were obtained from the animal facilities of CPqAM-FIOCRUZ, Recife. Experimental and
control groups were infected percutaneously with 90 cercariae (BH strain) shed from laboratory infected *Biomphalaria glabrata.*

**Drug administration.** The *C. parvum* was supplied by Instituto de Antibiéticos/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 LAWMAN et al. as modified by ABATH et al. 50 days after infection by *S. mansoni.* One tenth millilitre of a solution of Staphylococcus 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. 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:

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

- 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 60th 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 (x 10) that was calibrated through a stage micrometer. Only newly formed granulomatous lesions were measured, e.g. granulomas containing an eosinophilic mucicarum.

**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**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Leukocyte response*</th>
<th>PMN</th>
<th>MN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-infected/non treated</td>
<td>193.85 ± 80.11</td>
<td>2.78 ± 2.16</td>
<td></td>
</tr>
<tr>
<td>Infected/non treated</td>
<td>119.8 ± 7.83</td>
<td>2.36 ± 1.25</td>
<td></td>
</tr>
<tr>
<td>Prophylactic</td>
<td>125.47 ± 122.57</td>
<td>4.87 ± 3.92</td>
<td></td>
</tr>
<tr>
<td>Curative</td>
<td>71.47 ± 54.73*</td>
<td>2.01 ± 0.70</td>
<td></td>
</tr>
</tbody>
</table>

Each group was composed of ten mice.

* Means ± standard deviations;
PMN, polymorphonuclear; MN, mononuclear
* Statistically significant in comparison to the infected/non treated group.

**TABLE 2**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Adult worm recovery*</th>
<th>Protection %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (infected / non treated)</td>
<td>23.4 ± 8.82</td>
<td></td>
</tr>
<tr>
<td>Prophylactic</td>
<td>13.2 ± 6.64*</td>
<td>44</td>
</tr>
<tr>
<td>Curative</td>
<td>18.5 ± 6.02*</td>
<td>21</td>
</tr>
</tbody>
</table>

Each group was composed of ten mice

* 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 perivascular granulomas. However, differ-
ences 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 intesti-
tines, single eggs and perivascular 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 exquisitive type and seemed to be of a smaller size as
compared to those seen in the liver.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Granuloma*</th>
<th>Number</th>
<th>Mean Diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (infected/non treated)</td>
<td>23.5 ± 9.63</td>
<td>0.38 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>Prophylactic</td>
<td>13.7 ± 4.24</td>
<td>0.35 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>Curative</td>
<td>21.3 ± 7.04</td>
<td>0.35 ± 0.02</td>
<td></td>
</tr>
</tbody>
</table>

The granulomas were counted and measured in liver sections
of the same size, corresponding to ten mice.

\*Mean ± 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 produc-
tion and mobilization of PMN. A chemotaxis de-
defect was also demonstrated in other parasitic diseases,
such as Chagas's disease. The biological role of the de-
fect in chemotactic response found in experimental
schistosomiasis is not clear at the moment. The prophyl-
actic 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 portal mes-
enteric system. It is important to mention that no adhe-
sion 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 intraoperatively). The 44% protection reported in the present paper is comparable
to the immunity conferred by some schistosomiasis vac-
cine candidates. The reason why the prophylactic treat-
ment led 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 ac-
ivate macrophages (and consequently produce comple-
ment chemotactic fragments) and stimulate B cells. It
has been used in anticancer therapy as well as in several infectious diseases. Recently, it was shown that macrophages become activated to produce toxic nitric oxide and to kill schistosomula 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. There is evidence that these effector mechanisms may also operate "in vivo".

The effects of levamisole, another immunopotenti-
tator, on experimental schistosomiasis were differen-
t from C. parvum. 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 and, of course, the specific action
of each of them.

The present data suggest that the C. parvum treat-
ment before the S. mansoni infection is more efficient in
protecting against infection, probably due to the follow-
ing reasons: a) macrophage activation before infection;
b) greater efficiency in increasing the leukocyte re-
response; c) greater susceptibility of schistosomula.

Thus, macrophage activation and leukocyte chemotaxis
ACKNOWLEDGEMENTS

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