Brugia malayi in Mastomys natalensis: Effect of Immunostimulators on Establishment and Course of Infection*

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Abstract: Effect of three immunomodulators (IMS), muramyl dipeptide (MDP), Freund's complete adjuvant (FCA) and a newly synthesized sugar free immunomodulator (CDRI Comp. No. 84/201) on the establishment and course of Brugia malayi infection in Mastomys natalensis was evaluated. These immunomodulators were administered on three occasions (days -10, 0 and +15 of larval exposure) by subcutaneous route. All the three immunostimulators caused prolongation of prepatent period of the infection, the longest being observed with Comp. 84/201 (136.6 days) followed by MDP (120 days) and FCA (118.82 days). Comp. 84/201 and MDP treated animals revealed significantly suppressed microfilaraemia (P<0.01) and less percentage of worm recovery over untreated infected controls. It is, thus, surmised that establishment of filarial infection be affected by immunomodulators and a Comp. 84/201, a non-pyrogenic immunomodulator holds promise for the purpose.

Key words: Brugia malayi, Mastomys natalensis, Immunomodulators, Comp. 84/201

INTRODUCTION

Like many other parasitic infections, filariasis causes immunosuppression (Portaro et al., 1976; Ottesen et al., 1977; Grove and Forbes, 1979; Kwa and Mak, 1980; Mehta et al., 1980; Piessena et al., 1980a, b) which not only helps the parasite in sound anchorage in the host but may also adversely affect the therapeutic efficacy of drugs against parasites. It is therefore, of interest to examine whether such parasite-mediated immunosuppression can be reversed with immunomodulators thereby affecting the establishment and course of filarial infection.

In the present communication we report the results of studies on the immunomodulatory effect of Freund's complete adjuvant (FCA), muramyl dipeptide (MDP) (Lederer, 1986) and a newly synthesized analog of MDP, CDRI compound No. 84/201 (Haq et al., 1990) on establishment and course of Brugia malayi infection in Mastomys natalensis.

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MATERIALS AND METHODS

Experimental model

*B. malayi* in *M. natalensis* was used as experimental model. Six to eight weeks old male mastomys were exposed subcutaneously with 100 L₃ of *B. malayi* following the technique of Murthy *et al.* (1983).

Immunomodulators

As stated immunomodulators (IMS) used in the study were: FCA (DIFCO Laboratories, Michigan), MDP (Sigma Chemicals, U.S.A) and C.D.R.I. Comp. 84/201.

Synthesis of the lipopeptide 84/201

The synthesis of Comp. 84/201 was accomplished in the solution phase using DCC/HOBt (Konig and Geiger, 1970) and mixed anhydride (MA) (Anderson *et al.*, 1967) procedures for peptide bond formation. In the first instance, the dipeptide Boo-Gly-Lys (Z)-OMe (I) was obtained by condensing Boo-Gly (Schnabel, 1967) with Lys (Z)-OMe (Biossonnas *et al.*, 1958) in the presence of DCC/HOB alkaline hydrolysis of I gave the corresponding acid Boo-Gly-Lys (Z) (II) Which on treatment with lauryl amine in the presence of DCC/HOBt yielded Boo-Gly-Lys (Z)-NH.C₁₂H₂₅ (III). Cleavage of Z group from III by catalytic hydrogenation followed by treatment of the resulting amine with the mixed anhydride obtained from Z-D-Glu-NH₂ (Shealy *et al.*, 1968) afforded the tripeptide lauryl amide Boo-Gly-Lys-(Z-D-Glu-NH₂)-NH.C₁₂H₂₅ (IV). Removal of the Z group from IV by catalytic hydrogenation and treatment of the resulting tripeptide amine with Z-Ala-ONSu (Anderson *et al.*, 1964) gave the protected tetrapeptide Boo-Gly-Lys (Z-Ala-D-Glu-NH₂)-NH.C₁₂H₂₅ (V). Final deblocking of Boo and Z groups was achieved by treatment of V with HBr/AcDH and the lipopeptide 84/201 was obtained as a chromatographically homogeneous hydrobromide salt after repeated precipitation from MeOH/ether. It was characterised on the basis of its elemental analysis, HPLC and ¹³C NMR data.

Dose schedule of immunomodulators

Solutions of MDP and Comp. 84/201 were prepared in sterile distilled water and kept at -20°C. The method of Dietrich *et al.* (1983) was broadly followed in administration of multiple doses of IMS. Briefly, the doses tried were 250 or 500 μg per animal on three occasions (days -10, 0 and +15 of larval exposure). FCA was administered in 0.1 ml volume at the same time intervals. All the inoculations were given through subcutaneous route. Control animals received only vehicle.

At each dose level 5 to 10 animals in two replicated were used.

Assessment of effect of immunomodulators

Course of infection: Microfilaraemia of (20 mm³ of tail blood) infected animals was determined using conventional procedure on day 90 post inoculation (p.i.) and thereafter at 3-4 days interval till microfilaria (mf) appeared. Briefly, thick blood smear of 20 mm³ blood from each animal was made, air dried, dehaemoglobinized with tap water, stained with Leishman’s stain and mf were counted under microscope. Further monitoring of microfilariaemia was conducted at monthly interval upto day 180 p.i.
Recovery of adult worms: All the animals were sacrificed on day 180 p.i. under deep ether anaesthesia and worm burden was ascertained following the method of Murthy et al. (1983). Uterine contents of female worms were also examined according to the method of Lammler (1977).

Statistical analysis: Data were analysed by Mann Whitney ‘U’ test to determine the significance.

RESULTS

The effect of different IMS on the length of incubation period and worm recovery in mastomys following exposure to B. malayi infection has been appended in Table 1. Comp. 84/201 at both the dose levels (i.e. 250 and 500 μg) caused prolongation of incubation period over untreated controls which was statistically significant (P<0.01). The effect of MDP (at 250 and 500 μg) on incubation period was more or less similar (112.5 and 120.0 days respectively) to what was observed with FCA (118.82 days). Nevertheless, incubation period of B. malayi in untreated mastomys was 101.25 days.

The course of microfilaraemia of all experimental groups and controls has been expressed in Table 2 and a comparative analysis of the results has been depicted in Table 3. Microfilaraemia was very significantly suppressed (P<0.01) when Comp. 84/201 was used at 500 μg dose level. This suppression more or less persisted up to day 180 of observation period. Lower dose (250 μg) had lesser effect which was, however, significant (P<0.05). The effect of MDP at 500 μg dose level was observed to be highly significant (P<0.01) up to day 180 when compared to controls. The difference on microfilaraemia level as observed on days

<table>
<thead>
<tr>
<th>Immunomodulators</th>
<th>Dose/animal s/c×3</th>
<th>No. of animals used</th>
<th>Prepatent period in days Mean±S.D.</th>
<th>Percent recovery of adult worms (Mean±S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Male</td>
</tr>
<tr>
<td>Control</td>
<td>–</td>
<td>8</td>
<td>101.25±15.53</td>
<td>3.75±1.49</td>
</tr>
<tr>
<td>FCA</td>
<td>0.1 ml</td>
<td>11</td>
<td>118.82±14.00</td>
<td>4.36±2.42</td>
</tr>
<tr>
<td>MDP</td>
<td>250 μg</td>
<td>5</td>
<td>112.50±15.00</td>
<td>1.75±0.50</td>
</tr>
<tr>
<td>MDP</td>
<td>500 μg</td>
<td>5</td>
<td>120.00±30.00</td>
<td>2.00±0.71</td>
</tr>
<tr>
<td>CDRI Comp. 84/201</td>
<td>250 μg</td>
<td>8</td>
<td>128.57±14.64*</td>
<td>3.88±4.32</td>
</tr>
<tr>
<td>CDRI Comp. 84/201</td>
<td>500 μg</td>
<td>6</td>
<td>136.60±24.14*</td>
<td>1.83±0.98</td>
</tr>
</tbody>
</table>

*Significance at 5% level (P<0.05)
**Highly significance (P<0.01)
120, 150 or 180 p.i. was comparable to Comp. 84/201. However, the rate of increase in level of microfilaraemia at midpatency was more in animals treated at lower dose (250 μg) of MDP or Comp. 84/201. FCA also exerted suppression of microfilaraemia which was however comparable to effect of Comp. 84/201 at 250 μg dose level and was significant (P<0.05). Untreated controls showed sharp rise in microfilaraemia with variations at later stage of observation period (Table 2).

The effect of both MDP and Comp. 84/201 was very significant (P<0.01) at 500 μg dose level on establishment and recovery of adult worms (Table 1). A dose dependent effect

<table>
<thead>
<tr>
<th>Immuno-modulators</th>
<th>Dose/animal, s/c</th>
<th>Prepatent period in days (Mean±S.D.)</th>
<th>Mf count/20 mm³ (Median with range) on day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>90</td>
</tr>
<tr>
<td>Control</td>
<td>–</td>
<td>101.25±15.53</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0-96)</td>
</tr>
<tr>
<td>FCA</td>
<td>0.1 ml</td>
<td>118.82±14.00</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0-2)</td>
</tr>
<tr>
<td>MDP</td>
<td>250 μg</td>
<td>112.50±15.00</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0-2)</td>
</tr>
<tr>
<td>MDP</td>
<td>500 μg</td>
<td>120.00±30.00</td>
<td>0</td>
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<td></td>
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<td>(0-2)</td>
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<tr>
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<td>(0-2)</td>
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<tr>
<td>CDRI Comp. 84/201</td>
<td>500 μg</td>
<td>136.60±24.14</td>
<td>0</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>(0-10)</td>
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</tbody>
</table>

Table 3. Significance of course of microfilaraemia in treated M. natalensis over untreated control

<table>
<thead>
<tr>
<th>Experimental group (dose/animal)</th>
<th>Mf count/20 mm³ on day post larval exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>90</td>
</tr>
<tr>
<td>FCA (0.1 ml)</td>
<td>NS</td>
</tr>
<tr>
<td>MDP (250 μg)</td>
<td>NS</td>
</tr>
<tr>
<td>MDP (500 μg)</td>
<td>NS</td>
</tr>
<tr>
<td>Comp. 84/201 (250 μg)</td>
<td>**</td>
</tr>
<tr>
<td>Comp. 84/201 (500 μg)</td>
<td>**</td>
</tr>
</tbody>
</table>

NS Not significant (P<0.05)  
+ Animals were sacrificed.  
* Significant at 5 % level of significance (P<0.05)  
** Highly significant (P<0.01)
on establishment of adult worms was observed with Comp. 84/201. However with MDP both the doses tried had very significant effect (P<0.01). FCA also caused suppressed worm recovery but was less effective than MDP or its analog (Comp. 84/201). Nevertheless the effect was significant (P<0.05) over controls. Apparently on particular sex of adult filariids was proportionately affected when compared with counterparts of untreated controls (Table 1).

**DISCUSSION**

Suppression of host's immune response is a common phenomenon which is encountered in almost all parasitic diseases (Clayton, 1979) and may jeopardise control measures either by drugs or possibly with immunoprophylactic means. The present study showed that immunostimulators could affect establishment of filarial infections in host. The altered establishment and course of infection were revealed by enhanced incubation period, suppressed microfilaraemia and lowered percent recovery of adult worms. In general all the three immunomodulators used exerted effect though of variable intensities.

A critical analysis of relative efficacy of the three IMS on percent recovery of adult worms indicated that a 500 µg dose level MDP and its lipopeptide, Comp. 84/201 were superior to FCA and MDP was superior to Comp. 84/201 in suppressing the worm recovery at 250 µg dose level (P<0.01). However, at both the dose levels (250 and 500 µg) Comp. 84/201 significantly enhanced incubation period in comparison to MDP and FCA.

Microfilaraemia was also affected in all animals following administration of any of the three IMS. However effect of IMS on the course of microfilaraemia tapered off at the later stage of patency. Nevertheless at 500 µg dose level both MDP and Comp. 84/201 caused prolonged suppression of microfilaraemia. It is known that both MDP and FCA stimulate cell-mediated as well as humoral mediated immune responses (Chedid et al., 1977; Fevrier et al., 1978; Leclere et al., 1979; Lowy 1977; Roitt, 1980; Saxena et al., 1991) and that the cell-mediated immune response has been shown to play a major role in elimination of parasites (Haque et al., 1982; Subrahmanyam et al., 1978). Whether the observed effect of IMS on the establishment and course of B. malayi in M. natalensis is mediated by the stimulated immune response remains to be determined. Recently we (Murthy et al., 1992) found that FCA alone when administered to microfilaraemic animals would kill certain percentage of adult worms. It was also observed that FCA alone could significantly enhance antibody titre to filarial parasite. Klei et al. (1982) have demonstrated non-specific activation of cellular immune response following FCA alone injection in jirds. This was associated with changes in the lymphatics. We also found that IMS have on direct effect on the different stages on the parasite in vitro (unpublished observation). Thus an immunomodulation of the host rather than a direct effect on the parasite appears to be a distinct possibility. It is concluded that IMS: FCA, MDP and Comp. 84/201 inhibit the establishment and course of B. malayi infection in mastomys. Of the three immunomodulators Comp. 84/201 may hold promise as it was recently demonstrated to be non-pyrogenic (Haq et al., 1990).
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process for the synthesis of N-glycyl, N-(L-abanyl-D-isoglutaminyl)-L-lysyl-N-alkylamides possessing high 


291.

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