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Author(s): Misra, Shailja; Singh, D. P.; Chatterjee, R. K.

Citation: 热帯医学 Tropical medicine 30(1). p1-11, 1988

Issue Date: 1988-03-31

URL: http://hdl.handle.net/10069/4509

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Studies on Alteration in Antibody Level of Filarious Host Following Diethylcarbamazine Treatment

Shailja MISRA, D. P. SINGH and R. K. CHATTERJEE

Division of Parasitology, Central Drug Research Institute,
Post Box 173, Lucknow 226001 (U. P.), INDIA

Abstract: Sharp fall in antibody level of Mastomys natalensis infected with Litomosoides carinii occurred following treatment with diethylcarbamazine (DEC). Though reduced haemagglutinin titre returned to pretreatment level within 30 days, precipitin titre which disappeared totally did not show a come-back even upto day 50. The decrease in the levels appear to be due to increased consumption of antibody (more of IgG than IgM type) in reaction with dead and degenerated microfilarial products (antigens). This was substantiated by the facts that DEC did not alter antibody level specific to BSA or adult male parasites (thus lacking microfilariae). The concept of neutralisation of antibody in reaction with sudden massively released microfilarial products could also be established from experiments in which sharp fall in antibody occurred following administration of large amount (9 mg) of microfilarial (mf) antigen proteins in infected animals. It is concluded that fall in antibody titre following DEC therapy is due to quick death of microfilariae in large numbers and the drug has no direct effect on host's immune mechanisms.

Key words: Litomosoides carinii, Microfilariae, Diethylcarbamazine, Chemotherapy, Mastomys natalensis, Antibody

INTRODUCTION

Diethylcarbamazine is extensively used as chemotherapeutic agent against filariasis. However available informations indicate extension of its action to the immune machinery of treated host. Earlier Orange et al. (1968) reported DEC-induced inhibition of release of slow reacting substance of anaphylaxis in rodents. Later Katiyar et al. (1974) and Murthy et al. (1978) using respectively Wuchereria bancrofti and Brugia malayi infective larval antigens in skin test, demonstrated suppression of skin reactions in filarial patients following DEC treatment. Filaria-specific IgM and IgG antibodies were also reported to be suppressed when DEC was administered to infected rodents (Desowitz et al., 1978; Misra et al., 1982). Apart from effect of DEC on humoral immune status, cell-mediated immunity was also reported to be altered (Hewitt et al., 1981; Mistry et al., 1986). Apparently alterations in antibody level could possibly be due to effect of the drug on synthesis and/or release of
antibodies. Nevertheless quick effect of the drug on microfilariae (mf) may also contribute to altered immune status of treated host. The present study was therefore carried out to investigate the origin of altered antibody status following DEC treatment using *Litomosoides carinii* in *Mastomys natalensis* as working model.

**PLAN OF STUDY**

The modifying effect of DEC on antibody at different time intervals following treatment was initially evaluated in microfilaraemic mastomys following mite-induced *L carinii* infection.

To exclude the possibility of alteration due to interaction between materials from dead microfilariae following DEC therapy and circulating antibody, mastomys were infected by implantation of only adult male worms. DEC was administered only when specific titre was built up.

The next set of experiment was conducted to evaluate the effect of the drug in healthy animals immunised with homologous parasite antigen. DEC was administered in these animals only after the development of high antibody titre.

Investigation was also made on the effect of the drug on filaria-unrelated antibody status. Bovine serum albumin was used as immunogen.

Lastly to confirm the possibility of interaction between antigen proteins from dead mf following drug exposure and specific antibody, antigen immunised animals were challenged with homologous antigen at different dose levels and the antibody status was measured.

**MATERIALS AND METHODS**

**Infection:**

*Litomosoides carinii* infection maintained in *Mastomys natalensis* (Lammler et al., 1971) was used in the study.

**Assay of antibody**

Indirect haemagglutination (IHA) (Tanaka *et al.*, 1968) and precipitin (Chatterjee *et al.*, 1976) tests were performed to study the antibody status of hosts under various experimental conditions. The titre was expressed as log 2 titre.

a) **DEC treatment in mite-induced infection**

Batches of microfilaraemic mastomys (90-120 days old infection) were selected for DEC treatment at 25 mg/kg (base), i.p. ×5 days. Blood for sera were collected from the retroorbital plexus of each animal on day 0 and there-after on days 4, 10, 20, 30, 40 and 50 of start of treatment. Sera were also collected from identically infected untreated control animals.

b) **DEC treatment in male worm-implanted animals**

8 week-old six male mastomys were implanted peritoneally each with 10 live males.
30 days post implantation animals showing specific antibody titre were treated with DEC. Blood for sera was collected at same time intervals as mentioned earlier. Untreated implanted animals served as controls.

c) *DEC treatment in antigen-immunized animals*

Antigen was prepared from adult *L. carinii* of both sexes (Misra et al., 1982). Immunization schedule of each of 15 animals consisted of three doses (25 μg + FCA, 50 μg and 100 μg) being administered subcutaneously over a period of 30 days. Specific antibody was measured on day 8 of last immunizing dose. Five such animals were treated with DEC at 25 mg/kg (base), i. p. ×5 days while another 5 received treatment for 21 days. Rest of the animals were left untreated as controls. Sera were collected at usual time intervals.

d) *DEC treatment in BSA-immunised animals*

15 Albino rats (male, 12 week-old) were immunised with BSA (bovine serum albumin) using 3 doses (500 μg + FCA, 1 mg and 1.5 mg) spread over a period of 30 days. First and second group each consisting of 5 immunized rats received DEC (25 mg/kg) for 5 and 21 days, respectively and the rest were left as untreated controls. Sera were collected at the same intervals.

e) *Antibody titre following challenge to immunised animals with homologous antigens.*

A total of 15 infected mastomys (90-120 days old-infection) were used for the purpose. Five of these were administered intravenously with homologous soluble somatic antigen at 3 mg (*L. carinii* antigen protein) per animal whereas each of another 5 animals received 9 mg of antigen protein. The rest of infected animals served as controls.

Under similar conditions two groups each having 5 albino rats immunised with BSA, were challenged with homologous antigen protein (BSA) intravenously at 5 and 10 mg/animal respectively to groups 1 and 2. Blood samples for sera were taken on day 0, 1, 4, 10, 20 and 30 of BSA challenge. 5 untreated control animals (immunised with BSA) were also bled at identical intervals.

**RESULTS**

*DEC in L₅-induced infected animals*

Treatment of mastomys with DEC led to more than 90% fall in circulating microfilariae by day 4. However from day 10 onwards microfilaraemia started increasing steadily (Fig. 1). Nevertheless both IHA and precipitin antibodies also exhibited simultaneous sharp fall on day 4 of DEC treatment. Thus reduction in mf count coincided with fall in antibody level. The IHA titre which was 10 before treatment went down to 3 and 2 respectively on days 4 and 8. The suppressed IHA titre however recovered gradually from day 30 onwards and reached to 7 on day 50. In contrast precipitin titre which was 6 on day 0 (just before treatment) disappeared within 4 days of start of DEC therapy and did not reappear even upto day 50 of observation period. Untreated infected animals showed progressive rise in antibody titre.
Effect of DEC on antibody level in homologous antigen-immunised animals

Animals immunised with soluble somatic antigen of *L. carinii* did not reveal any suppression in IHA or precipitin antibody titre even when the treatment was extended upto 21 days. Similar observation was made in animals which were immunised with BSA and treated with DEC (Table 1).

![Graph showing effect of DEC on antibody level](image)

**Fig. 1.** Effect of DEC on IHA and precipitin titres of *Mastomys* infected with *L. carinii*.  
- - - Precipitin titre (Treated); - - - Precipitin titre (control)  
- - - IHA titre (Treated); - - - IHA titre (control)  
- - - % mf/5 cmm of blood

**Table 1.** Effect of DEC on antibody titre of rodents immunised with filarial antigen / BSA

<table>
<thead>
<tr>
<th>No. of animals</th>
<th>Immunogen</th>
<th>Host</th>
<th>DEC (base) treatment</th>
<th>Observation period</th>
<th>Effect on antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>L. carinii</em> (adult antigen)</td>
<td><em>M. natalensis</em></td>
<td>25mg/kg x 5 days, i.p.</td>
<td>Day 1 to day 40 post treatment</td>
<td>No effect, No effect</td>
</tr>
<tr>
<td>5</td>
<td>- do -</td>
<td>- do -</td>
<td>25mg/kg x 21 days, i.p.</td>
<td>- do -</td>
<td>- do - - do -</td>
</tr>
<tr>
<td>5</td>
<td>- do -</td>
<td>- do -</td>
<td>-</td>
<td>- do -</td>
<td>- do - - do -</td>
</tr>
<tr>
<td>5</td>
<td>BSA</td>
<td>Albino rat</td>
<td>25mg/kg x 5 days, i.p.</td>
<td>- do -</td>
<td>- do - - do -</td>
</tr>
<tr>
<td>5</td>
<td>- do -</td>
<td>- do -</td>
<td>25mg/kg x 21 days, i.p.</td>
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<td>5</td>
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</tbody>
</table>
**Effect of DEC on antibody level in male implanted animals**

Animals implanted with adult male worms developed both IHA and precipitin antibody (Fig. 2). However no alteration in antibody titre in these animals could be recorded.

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**Fig. 2.** Effect of DEC on IHA and precipitin titres of *Mastomys* implanted with male worms (*L. carinii*).

- • IHA titre (Treated); • IHA titre (control)
- x—x Precipitin titre (Treated); x—x Precipitin titre (control)

**Fig. 3.** Effect of homologous *L. carinii* antigen (i. v.) on IHA titre of *Mastomys* infected with *L. carinii*.

- • 3 mg adult Ag; ■ ■ 9 mg adult Ag; • • Control
following treatment with DEC.

*Effect of homologous antigen challenge in sensitized host*

Homologous (*L. carinii*) antigen was administered intravenously in infected host to mimic the situation created following death of microfilariae after DEC therapy. It was found that a single massive dose of antigen (9 mg of *L. carinii* antigen protein/animal) could significantly suppress specific antibody. The IHA titre of infected animals which was 11 decreased to 6 within 24 hrs of challenge. However suppressed titre returned to pretreatment level on day 20. Challenge with lower amount of antigen protein (3 mg/animal) however caused significant boost in antibody titre from day 4 onwards reaching to a maximum on day 10. Similar observations were made with precipitin titre following homologous antigen challenge. A quantum of 9 mg antigen protein caused suppression of precipitin antibody titre within 24 hrs but went up from day 4 (Fig. 3 and 4).

*Effect of DEC on BSA specific antibody level*

Albino rats immunized with BSA when treated with DEC did not reveal any alteration in antibody level (Table 1). However a single massive challenge of immunized animals with homologous immunogen (BSA) resulted into total elimination of IHA and precipitin titres within 24 hrs. Nevertheless recovery from suppression occurred gradually (Fig. 5 and 6).

![Fig. 4. Effect of homologous *L. carinii* antigen (i. v.) on precipitin titre of *Mastomys* infected with *L. carinii*.](image-url)

- ●● 3 mg Ag i. v;
- ■■ 9 mg Ag i. v;
- ○● Control
Fig. 5. Effect of BSA (i. v.) on IHA titre of rats immunised with homologous protein.  
- - 5 mg BSA i. v; - - 10 mg BSA i. v; - - Control

Fig. 6. Effect of BSA (i. v.) on precipitin titre of rats immunised with homologous protein.  
- - 5 mg BSA i. v; - - 10 mg BSA i. v; - - Control
DISCUSSION

The present study showed total abolition of precipitin and 70% reduction of HA antibodies within 4 days of DEC treatment approximately coinciding with death and disintegration of microfilariae. To investigate further the role of dead microfilarial products as the plausible cause of reduction in antibody level, only male worms-implanted animals were treated with DEC and no change in antibody level occurred. Thus involvement of microfilaria appears to be necessary in suppression of antibody level.

The observed alteration in antibody level following DEC therapy may also be due to suppressive effect of drug on immune mechanisms including synthesis and/or release of antibody by the competent cells. However this hypothesis would not be tenable as animals immunised with filarial antigen or BSA when treated with DEC did not reveal any change in specific antibody level. Earlier Saxena et al. (1983) observed no change in antibody responses to Ascaris, TAB vaccine and red cells following DEC therapy.

Under living condition circulating microfilariae are camouflaged by incorporating host proteins (albumin) on their surfaces (Forsyth et al., 1981; Maizels et al., 1984; Philipp et al., 1984). DEC unmasks the surface of microfilariae so as to expose it to host's immune system (Gibson et al., 1976; Piessens and Beldekas, 1979; Hammerberg, 1985). The exposed surface reacts with antibody forming antigen-antibody complexes (Hawking, 1978). This is further evidenced by the fact that DEC is ineffective against microfilariae in animals deprived of specific antibodies (Kobayashi et al., 1969). Perhaps filaria-specific antibody is increasingly consumed in reaction with 'exposed' as well as dead and disintegrated microfilarial products. However, the findings of Palumbo et al. (1978) are significant in this context. They observed that DEC stimulates adult female worms to release a "free" antigen which complexes with pre-existing antibodies.

Increase in circulating antigen following successful therapy is known in filarial infections. Cotton rats with L. carinii infection when treated with DEC or Suramin resulted in increase of free circulating antigen (Carme et al., 1982). Forsyth et al. (1984) also observed significant rise in specific antigen level following benzimidazole treatment of cattle infected with Onchocerca gibsoni. Increased level of antigen may act as immunosuppressant and/or as stated earlier, may combine with specific circulating antibody to form immune complexes. Most of the immune complexes in blood are, however, rapidly cleared by mononuclear phagocyte system especially the Kuffer cells (Mannik and Arand, 1971; Weigle, 1961). It is less likely that reduced level of antibody would be due to immunosuppressive effect of overwhelming antigenaemia as fall in antibody level was rather quick (i.e. total elimination of serum precipitin titre within 4 days of treatment).

The hypothesis of neutralization of serum antibody by increased antigen level following sudden death of microfilariae in large numbers was further established when BSA or parasite antigen immunised animals were intravenously challenged with homologous proteins. From such studies with L. carinii antigen, it was evident that the dose of 3 mg enhanced antibody level whereas at 9 mg there was significant fall in IHA titre (from 11
to 6) within 24 hours. The fall in titre in DEC treated microfilaraemic animals was however not as faster as that of antigen-immunised animals challenged with homologous antigens as in the former case, it was time consuming for the circulating microfilariae to be killed and disintegrated in the host.

In the present study HA antibody of treated animal started reappearing from day 20 onwards, however precipitin titre remained suppressed for longer period and recovery was very slow. It was also the fact that IHA titre never disappeared totally following application of DEC. It is difficult to explain these phenomena. Perhaps IgG antibody was more significantly consumed than IgM type of antibody. Nevertheless it is obvious that total disposal of dead microfilarial bodies is a time consuming affair. The IHA titre in the present study was also measured after β-mercaptoethanol treatment of sera. Boreham and Atwell (1983) estimated immunoglobulins of DEC-treated dogs infected with Dirofilaria immitis and did not find any alterations in total IgM content of serum. However they did not estimate filaria-specific antibody following DEC treatment.

Thus in conclusion fall in serum antibody (IHA and precipitin titre) level specific to filaria following DEC treatment is not due to the effect of the drug on immune machinery of host but due to neutralization of already present circulating antibody by antigens derived from dead mf. The altered antibody level slowly comes back to pretreatment level perhaps with the removal of immune complexes and fresh build up of microfilaraemia.

ACKNOWLEDGEMENTS

Sincere thanks are due to Dr. M. M. Dhar, FNA, Director, Central Drug Research Institute, Lucknow for his keen interest and constant encouragement in carrying out this work. Financial assistance by the Indian Council of Medical Research in the from of Senior Research Fellowship to one of the authors (D. P. S.) is gratefully acknowledged.

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