Summary and Conclusions
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CHAPTER- 6

Lymphatic filariasis is primarily a disease of the poor because of its prevalence in remote rural areas and in disfavored peri-urban and urban areas. LF is caused by nematode parasite species *Wuchereria bancrofti*, *Brugia malayi* and *B. timori*. It is transmitted through mosquitoes. Globally, LF is thought to be second leading cause of permanent and long term disability. Some 120 million people are infected worldwide, and the disease is endemic in more than 80 countries and territories. More than 90% of cases with *W. bancrofti* and 10% of cases with *B. malayi* are recorded. The spectrum of lymphatic filariasis is analogous to leprosy. Microfilaria carriers with no signs and symptoms represent one pole and patients with elephantiasis and chronic lesions represent the other pole.

Several strategies are currently being tried for control and eradication of filariasis but the main emphasis continues to be on vector control and chemotherapy. DEC, IVM and ALB are the antifilarials used in chemotherapy. Recently, the World Health Organization (WHO) in collaboration with GlaxoSmithKline launched a global LF elimination program with a drug combination approach consisting of treatment schedules with ALB + DEC or IVM. However, the most intriguing feature of the infection is its unpredictable recurrence after treatment. The reasons for this are not clear. For instance, it is not known whether a) the altered immune responses of the host following treatment with antifilarial(s) are able to provide conducive environment for re-infection, b) individuals become more vulnerable to acquiring infection or become resistant to re-infection after a course or repeated courses of treatment, and c) prior exposure to antifilarial(s) alter the biology/infectivity of parasite. Unfortunately there have been no systematic studies to answer these
questions. The present study was therefore, aimed at investigating alterations in immune responses of the *B. malayi*-infected host after treatment with antifilarials and after re-infection of the treated host. This will help understanding the possible reasons involved in the recurrence of infection or resistance to therapy in human filariasis.

The present study was carried out using *M. coucha-B. malayi* model comprising the main groups (Table as below): infected animals, infected treated animals, infected treated re-infected animals, infected re-infected and age and sex matched normal infected animals to see the effect of re-infection in antifilarial treated animals on the modulation of acquired immunity with particular reference to parasitological, physiological and immunological responses of the host.

**Selection and grouping of animals**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of animals</th>
<th>Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>16</td>
<td>Infected (Inf)</td>
</tr>
<tr>
<td>II</td>
<td>8</td>
<td>Infected Treated (Inf-T)</td>
</tr>
<tr>
<td>III</td>
<td>8</td>
<td>Infected Treated-Reinfected (InfT-R)</td>
</tr>
<tr>
<td>IV</td>
<td>8</td>
<td>Infected-Reinfected (Inf-R)</td>
</tr>
<tr>
<td>V</td>
<td>16</td>
<td>Normal (N)</td>
</tr>
<tr>
<td>VI</td>
<td>8</td>
<td>Normal-Treated (N-T)</td>
</tr>
<tr>
<td>VII</td>
<td>8</td>
<td>Normal Treated Infected (NT-I)</td>
</tr>
<tr>
<td>VIII</td>
<td>8</td>
<td>Normal-infected (N-I)</td>
</tr>
</tbody>
</table>

DEC at 150 mg citrate/kg or ALB at 50 mg / kg were given through i.p. and s.c. route, respectively. The agents were administered five consecutive days. Fresh solution was prepared daily before administration.

Infected or normal treated animals were exposed to L₃ on day 30 since the start of the treatment.

Various parameters like parasitological, blood cell counts and immunological (humoral, cell proliferation and cytokines) responses of the
Summary and Conclusions

host before treatment, one month post-treatment and one-month post-larval (L3) inoculation (= two month post-treatment) were carried out.

The antibody response of pre- and post-DEC/ALB treated and subsequently re-infected animal sera samples were analyzed qualitatively with western blot of BmA and BmMf and Sephadex G-200 chromatographic fractions of BmA. Experiments were also carried out to correlate DNA integrity using comet assay of lymph node and spleen cells of *M. coucha* harbouring early, mid and chronic stages of *B. malayi* infection and cell proliferative response to filarial antigen and mitogen of the host.

The salient findings of the present study are as follows:

In contrast to control animals (Inf) a marked reduction in Mf count (80%) was found in Inf-T animals on day 7 since the start of the treatment, which thereafter remained suppressed till the day of sacrifice. The level of microfilaraemia in InfT-R animals increased within a week after challenge with L3, and crossed the pre-treatment level by day 63. However, the level of Mf in Inf-R animals did not increase and remained comparable with the pre-treatment level throughout the observation period.

DEC treatment failed to kill adult worms. The percent recovery of developing stages of larvae from InfT-R animals was higher as compared to their counterparts Inf-R and N-I animals indicating that DEC treatment might be responsible for such enhancement in worm recovery.

An inverse correlation between microfilaraemia and eosinophilia was found. Decrease in eosinophil counts was accompanied by removal of Mf from the circulation.

Treatment with DEC in Inf-T animals caused increase in IgG levels directed against BmA, BmA-I and BmMf of which anti-BmA antibody levels remained almost unchanged till the day of sacrifice. In InfT-R animals the anti BmA and anti-BmMf-I antibody levels were comparable.
Summary and Conclusions

to levels of Inf-T animals, whereas in the Inf-R group, anti-BmA-I and BmMf IgG levels increased and the IgG levels against rest of parasite preparations decreased significantly.

Treatment with DEC did not alter the anti-BmA, anti-BmA-I, BmMf and BmMf-I IgM levels in any of the animals except age and sex matched animals of group N-I.

Treatment restored the proliferative response of cells of infected animals to Con-A and BmA-I, which however suppressed after reinfection.

While DEC treatment in infected animals caused decrease in BmA and LPS stimulated IL-6 production, TNF-α production was found to be increased. In InfT-R animals BmA induced IL-6 production was further decreased but no further increase in the level of BmA induced TNF-α production was observed in these animals. In contrast LPS stimulated cells produced significant increase in TNF-α production.

DEC treatment caused decrease in BmA induced IL-10 production in Inf animals. In InfT-R it further decreased. BmA induced IFN-γ production was increased after treatment, which was again increased (more than 3 times) after re-infection.

Treatment with ALB in Inf animals resulted complete clearance of microfilaraemia and worms. Interestingly neither InfT-R nor NT-I animals showed developing stages of the parasites indicating prophylactic potential of ALB.

The decrease in eosinophil counts in response to ALB treatment was less as compared to the effect of DEC. Re-infection in treated animals failed to increase the TLC and eosinophil counts.

Treatment with ALB caused significant and gradual decrease in IgG directed against BmA, BmA-I, BmMf and BmMf-I till the day of sacrifice. Re-exposure with L3 in treated animals (InfT-R) caused further decrease in
Summary and Conclusions

anti-BmA, BmMf, BmMf-I IgG levels. In Inf-R animals, IgG levels increased significantly as compared to InfT-R animals.

Treatment with ALB showed significant decrease in IgM levels directed against BmA, BmMf and BmMf-I in infected animals. Re-exposure of infected treated animals (InfT-R) caused significant decrease in anti- BmA and BmMf-I IgM antibody levels. However levels of anti-BmA-I and anti-BmMf IgM in InfT-R animals remained unaltered compared to Inf-T.

Inf-R animals showed increase in anti-BmA and anti-BmMf-I IgM antibodies.

Like DEC, ALB also restored the proliferative response of both lymph node and splenocytes to all the stimulants, although the response towards Con-A was significantly high. Re-infection failed to further alter the proliferative response of the spleen cells or lymph node cells to the stimulants when compared with Inf-T group. Interestingly, cells of Inf-R animals showed significantly decreased proliferative responses to the stimulants.

ALB treatment caused significant increase in IL-6 production in infected animals. However in InfT-R animals the IL-6 production was decreased when compared with value obtained in Inf-T animals but is comparable to the level of Inf animals.

Treatment (ALB) did not affect the TNF-α production in infected animals but in InfT-R animals the production decreased.

IFN-γ production in ALB treated (Inf-T) animals was upregulated significantly, which however decreased after re-exposure (InfT-R animals).

ALB treatment caused increased IL-10 production in Inf animals. However in InfT-R animals the level of BmA stimulated IL-10 production remained almost same when compared with Inf animals.
In Inf-R animals, BmA stimulated IL-6 and TNF-α production was comparable to Inf animals. Whereas re-infection up-regulated BmA stimulated IFN-γ production in infected (Inf) animals, BmA stimulated IL-10 production was found to be decreased.

The present findings on the effects of DEC in *B. malayi* infected *M. coucha* are in accordance with the reported observations in bancroftian patients treated with DEC. The following are the inferences made from the above results of the present study. Firstly, since Th2 cells produce IL-4 and IL-10, which were reported to facilitate parasite survival and that the magnitude of response correlates with the severity of disease, the increase in IL-10 observed in InfT-R group in the present study clearly demonstrates that DEC treatment prior to re-infection facilitates the 'take'/establishment of the infection. Second, a quantitative relationship between the raised TNF-α levels and the development of re-infection was demonstrated for the infected or infected treated animals. Third, while DEC decreased IL-10 production, ALB elevated IL-10 production. This suggests that the adult antigens available after ALB induced adult parasite killing, stimulate IL-10 production. This is further supported by the observation that IL-10 production is not stimulated by DEC treatment since DEC is not an efficient adulticidal agent. Fourth, IL-6, TNF-α and IFN-γ increased significantly after treatment with DEC in normal uninfected animals while IL-10 remained decreased. The significance of this observation to establishment of or resistance to re-infection is not readily evident, but the effect is clearly a direct effect of DEC rather than DEC 'opsonized' parasite antigen effect since no antigen is available in these animals. Fifth, apparently elevated levels of IFN-γ in re-infected animals correlated with the lesser number of the parasite establishment. This finding is in contrast to DEC treatment in infected animals, which facilitated the development of re-inoculated *L*₃ as evidenced by development of more number of parasites.
Sera of infected DEC treated animals reacted with a lesser number of bands of BmA and BmA-I. The range of reactive bands increased after re-infection showing strong reactivity with 32 and 45 kDa molecules of ether BmA and or BmA-I. Though Inf-R sera also identified lesser number of bands they reacted strongly with 22 and 28 kDa molecules of BmA and or BmA-II.

Sera of all the categories of animals reacted either moderately or weakly with 38 kDa and below of BmA-II antigen.

Sera from Inf-T animals (ALB treatment) reacted with 6.8 - > 160 kDa BmA antigen molecules. InfT-R sera reacted strongly with 62 kDa molecule of BmA and BmA-I was strongly reactive.

Sera of Inf-R animals reacted strongly with 22 and 28 kDa molecules of BmA.

The level of DNA damage in the splenocytes and lymphocytes of *M. coucha* (uninfected and *B. malayi* infected) was assessed on the basis of migration of DNA. The chronically infected animals showed DNA damage in both splenocytes and lymphocytes, however, 75% of animals harbouring mid stage of infection with Mf in the peripheral blood showed substantially low damage in DNA of splenocytes only. Interestingly, animals of early stage of infection with no peripheral Mf showed no damage in DNA of splenocytes or lymphocytes. Apparently there was a direct correlation between extent of DNA damage and duration of exposure of infection. Further DNA damage appeared to be correlated with the proliferative response of splenocytes or lymphocytes of animals to filarial extract and mitogen.

Thus from the present study it is concluded that

- In ALB treated and reinfected animals, increased the BmA specific CMI, which may be correlated with absence of (resistance to) re-infection.
Summary and Conclusions

- Slow and gradual decrease in filarial specific IgG in ALB treated (Inf-T) and no further appearance of IgG after re-infection correlated with the absence of re-infection.

- A prompt increase in filarial specific IgG in infected DEC treated (Inf-T) animals and no further alteration in IgG levels but re-suppression in cell proliferation after re-infection supported by the presence of developing stages of re-infection.

- Whereas 62 kDa band recognized by sera of ALB treated reinfected animals was correlated with development of resistance against re-infection, 32 and 45 kDa bands recognized by sera of DEC treated reinfected animals correlated with the development of re-infection. However, 22 and 28 kDa bands were identified with sera of Inf-R animals, which showed establishment of lesser number of worms. These antigens may be important for development of strategies for treatment/control modalities of the disease.

- DNA damage in splenocytes/lymphocytes of animals harbouring chronic stage of *B. malayi* infection correlated hyporesponsiveness of cellular proliferative response of the host.

Thus the results of the present study indicate that while ALB treatment may facilitate development of resistance against re-infection, DEC treatment may enhance recurrence of infection,