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15. Molecular monitoring of Vibrio cholerae in hospitalized diarrhoeal patients and aquatic environment in Puri district of Orissa.
1.1 Lymphatic filariasis in young children: an immunological prospective

Objectives:

1. To detect pre-patent infection through IgG4 and circulatory filarial antigen assay.

Background information:

Children (n=565) below 15 years of age of filarial endemic villages of Khurda district of Orissa were recruited in the study. Microfilaria prevalence rate among children was observed 6.54%. Presence of circulating filarial antigen (CFA) was determined using Og4C3 test kit. About 32% of the children were found CFA positive. Majority of CFA positivity (25%) was detected in asymptomatic amicrofilaraemic children. Infection free children (antigen negative) were checked whether these subjects were exposed to the infection or not. About 95% IgG positivity to S. digitata antigenic extract was observed indicating that these children were well exposed to filarial infection. Prevalence of IgG antibodies to filarial antigens (Setaria digitata antigen and Dssd1) in different age classes of endemic children was determined. More than 50% children were observed IgG positive by age of 5 years.

Results:

In order to check the transplacental transfer of filarial antigen and antibody, cord blood along with the maternal blood samples were collected from Khurda (district hospital), an area endemic for W. bancrofti infection. IgG and IgM antibodies to Setaria digitata antigens were determined in both maternal and cord blood samples (n=154). IgG positivity of 85% and 43% were observed in maternal and cord blood respectively. Similarly, IgM positivity rate of 55% was noticed in maternal blood samples. Only one cord blood sample was found positive for the same antibody. About 35% of mothers were found IgG positive to Dssd1 antigen vs. 10% in cord blood samples.

Presence of circulating filarial antigen (CFA) was checked in maternal and corresponding cord blood samples. About 56% of mothers were found antigen positive whereas 18% of cord blood samples were found positive for antigen.

1.2 Immunochemical characterization of filarial Glutathione S-transferase and its protective potential in experimental filariasis.

Objectives:

1. To determine recognition pattern of anti-Glutathione-S-transferase (GST) antibodies (SDS-PAGE and immunoblotting) in filarial sera.
2. To determine the cytokine responses specific to GST in filariasis.
3. To evaluate the protective potential of GST to clear microfilariae in experimental infected animal.

Progress:

Glutathione-S-transferase (GST) are essentially detoxification enzymes helps in parasite survival against host induced damage. These enzymes have been used as vaccine candidate antigens in schistosomiasis, fascioliasis and in chaga’s disease. In this study we have purified GST from adult S. digitata (the cattle filarial parasite) through Glutathione Agarose column to evaluate such role in human filariasis. IgG and IgM antibodies to Glutathione-S-transferase were determined in individuals living in areas endemic for W. bancrofti infection. About 90% of asymptomatic microfilaraemics (AS) and chronic filariasis (CP) patients were IgG positive.
compared to about 20% positivity in endemic normal (EN) individuals. IgM levels did not differ much among filarial groups such as EN, AS and CP groups. Seropositivity of 90% were observed in CP and AS group of patients. About 50% positivity was observed in endemic normals.

1.3 Innate immune recognition of filarial parasites by phagocytes

Objectives of the proposal:

1. To identify and characterise the molecular moieties from filarial parasites binding to murine antigen-presenting cells (APCs)
2. To identify murine APC surface receptors recognising filarial molecular targets
3. To study the signal transduction contributions of the newly identified APC receptors in mediating phagocyte activation in response to filarial parasite targets
4. To evaluate the roles of the identified receptor-ligand pairs in regulating filarial clearance in vivo

Progress:

Microfilarial clearance is poorer in DBA/2 mice despite having normal levels of Btk:

As mentioned in the last annual report we had observed that two mouse strains with the same MHC haplotype [H-2d] namely BALB/c and DBA/2 showed differential response to mf proteins as well as the ability of mice to clear mf from circulation. This year we report further characterisation of these findings.

We began with the assumption that if there is a difference in the mf clearing ability *in vivo*, it is likely that a strain showing better clearance is more likely to be a candidate to possess a receptor for mf. We used BALB/c and DBA/2, both of H-2d haplotype and BALB/c is known to clear mf rapidly. *S. digitata* mf were given [5x10^5 per mouse] intraperitoneally (i.p.) and microfilaraemia was tracked over a period of time.

Figure 1 shows that within two weeks DBA/2 mice show very high numbers of mf/10 ^µl of blood, while in BALB/c mice no mf are detected. After about 30-40 days of injection microfilaraemia subsides in DBA/2 mice. We have published a similar finding earlier attributable to Bruton’s tyrosine kinase [Btk]. In xid mice, which are deficient in functional Btk due to a mutation in plekstrin homology domain, there is high microfilaraemia as compared to the wild type [WT] CBA/J mice [1]. So as preliminary evidence we looked at the levels of Btk in the peritoneal macrophages of these mice. Whether activated *in vitro* with LPS or not, cell lysates of elicited peritoneal macrophages from BALB/c and DBA/2 mice show comparable levels of Btk signal in the Western blot as shown in Figure 2. The antibody used does not detect functionality or otherwise of Btk, however, it shows that the levels of the protein present in cells are comparable.

Our earlier data had indicated the macrophage effector functions to be contributing to the differences in mf clearance observed rather than any major impact of the adaptive immune system. Hence we tried to characterise the effector functions and the cell-surface receptors, which might trigger the effector functions including TLR receptors.

**Analysis of effector functions in macrophages from BALB/c and DBA/2 mice:**

We analysed effector functions of macrophages with two aims in mind. One, whether microfilarial proteins affect macrophage functions differentially in the two strains of mice and two, whether known TLR-ligation on macrophages is bringing about differential outcome in the two strains of mice.

Peritoneal macrophages from BALB/c and DBA/2 strains of mice were cultured in presence of various doses of *S. digitata* mf extract, *B. pahangi* mf extract or purified AgW antigen for 48 hours and the ability of these macrophages to produce nitric oxide was measured by nitrite accumulation in the supernatant by Greiss reaction. Figure 3 shows that macrophages from DBA/2 mice which clear mf more slowly produce less of nitrite in response to all mf antigens tested, as compared to macrophages from BALB/c mice. Since the possibility of LPS contamination in mf extracts cannot be ruled out, we used polymixin-B to counter LPS effects...
and estimated nitrite accumulation in a similar assay – in presence and absence of polymixin-B. Figure 4 shows that when high levels of nitrites were produced on stimulation, presence of polymixin-B could bring about reduction in nitrite production to near background levels as in BALB/c macrophages. However there was no reduction in nitrite producing ability of DBA/2 macrophages in presence or absence of Polymixin-B. More importantly, responses to a purified mif antigen AgW were also inhibited by polymixin-B in BALB/c macrophages [Fig 4] indicating that AgW may also be using an LPS-mediated activation pathway to produce effector molecules in macrophages. This raised a possibility of BALB/c and DBA/2 macrophages expressing a partially non-overlapping set of TLRs.

We used LPS, a ligand for TLR-4 and peptidoglycan [PG], a ligand for TLR-2 to stimulate macrophages from the two strains of mice. Interferon-γ [IFNg] is a known activator of macrophages, which works independent of TLRs and hence was used as a positive control. Data in Figure 5 show DBA/2 macrophages consistently produce less nitrites in all the assays, though with IFNg and PG as stimulators the differences in nitrite production from macrophages of the two strains are only marginal. In contrast, on LPS activation, macrophages from DBA/2 mice produce much less nitrite than those from BALB/c mice. The results have been consistent and BALB/c macrophages produce the same amount of nitrites with 30-50 fold lower dose of LPS. We have also used a TLR-3 ligand poly-l,poly-C to look at the nitrite producing capacity of macrophages and in preliminary experiments find that poly-IC is more potent in stimulating BALB/c macrophages than the DBA/2 macrophages [data not shown].

We have begun to look at another effector function of macrophages namely production of reactive oxygen intermediates [ROI] and ability to secrete various cytokines. In preliminary experiments we have looked at the ability of macrophages to produce ROI in response to LPS over a 6-hour period. The fluorescence of the dye diaminofluoresceinediacetate [DCFDA] added in culture is detected by fluorimeter periodically to estimate comparative levels of ROI produced. Figure 6 shows that macrophages from BALB/c mice produce higher levels of ROI as compared to DBA/2 macrophages in response to LPS indicating that both the effector functions – production of RNI and ROI go hand in hand as far as the differences in the two sets of macrophages are concerned.

Analysis of TLR-4 in macrophages:

We next decided to focus on TLR-4 to see if the levels of TLR-4 are different in the two strains of mice. Peritoneal resident cells [PRCs] and elicited cells [PECs] from BALB/c and DBA/2 mice were used to look for TLR-4 expression by flowcytometry. Figure 9 (A, B, C) shows the staining pattern when phycoerythrin [PE]-coupled anti-TLR-4 antibody was used. BALB/c PRCs show a good uniform staining 30-fold above the background stain. In contrast, DBA/2 PRCs show only a subset positive for TLR-4. The intensity of this staining is also much lower than that observed in BALB/c PRCs [Fig 9a].

On BALB/c PECs the staining intensity went down rather than up and only a subset showed positivity [Fig 9b]. The levels and numbers were even lower in DBA/2 PECs. On ligand binding TLR-4 can be internalised. In intestinal epithelial cells TLR-4 has been shown to be present intracellularly in association with its ligand LPS. Whether that can be the case in macrophages and whether such a receptor would be actively functional is not known. However, other family members of TLR are present intracellularly as well. Hence we looked at the total TLR-4 levels in these macrophages after cell permeabilisation. As compared to Figure 9b, which shows different surface TLR-4, levels in BALB/c and DBA/2 macrophages, total TLR-4 levels after permeabilisation are comparable as shown by superimposing curves in Figure 9c. The next question was whether addition of TLR-4 ligand in culture would alter these levels.
LPS was added for 2 hours in adherent PEC cultures and surface as well as total TLR-4 levels in macrophages were analysed by flow cytometry. Figure 10a shows surface staining whereas Figure 10b shows total staining for TLR-4. LPS-activated BALB/c PECs show much higher levels of surface TLR-4 as compared to DBA/2 PECs, and both show upregulation in levels on LPS treatment, indicating that signalling through TLR-4 may result in its increased surface expression.

In order to see whether macrophages from DBA/2 are deficient in cell surface expression of all TLRs we used a TLR-2 detecting antibody. Similar to Figure 10, staining before and after permeabilisation was carried out in LPS stimulated or unstimulated macrophages. Figure 11 shows that in macrophages from both the strains TLR-2 levels were comparable in every situation. Thus, the differences observed may be TLR-4 specific.

The data show that, unlike TLR-4 mutant mice C3H/HeJ, macrophages from DBA/2 mice have normal levels of TLR-4 but unlike macrophages from BALB/c mice a significant proportion of TLR-4 remains intracellular. Further significance of these findings needs to be evaluated.

**Analysis of filarial antigen binding to mouse macrophages and human monocytes:**

The above studies on filarial antigen induced intracellular signalling in mouse macrophages (presumably through TLR4 as shown above) necessitated the search for identification of filarial antigens that specifically bind to host cell surface molecules. Biotinylated adult filarial antigens bound significantly to human peripheral blood monocytes (Fig 12). This binding could be consistently demonstrated on monocytes of nine normal human subjects (Fig 13). More significantly the specificity of this binding was shown by competitive inhibition with non-biotinylated filarial antigens (Fig 14). This suggests the presence of specific filarial recognition molecules on normal human monocytes. Similar molecules were completely absent on human lymphocytes.

Unlike human lymphocytes, recognition molecules for filarial antigens could be recognized in normal mice. About 15-20% of lymphocytes in spleen of three different mouse strains CBA/J, Balb/C and C3H/HeN bound filarial antigens specifically (Fig 15). About 35% of the mouse macrophages of the three strains bound filarial antigens (Fig 16). There was no significant difference between the three strains of mice. We now propose to identify 1) host receptors in wild type as well as TLR-4 deficient macrophages that bind filarial antigens and also identify 2) the nature of parasite antigen(s) that bind to human monocytes and macrophages. It is also proposed to use mouse strains deficient for different genes such as IFN-g, IL-10, iNOS, ICAM-1, Btk, IL-4 etc. to study their respective macrophages for binding filarial antigens.

**Fig. 1.**

![Graph showing mf/10uL of Blood over Days post mf injection for BALB/c and DBA/2](image-url)
Fig. 2.

75 KD

Fig. 3.

Fig. 4.

BALB/c [-Pol] ■
BALB/c [+Pol] □
DBA/2 [-Pol] ●
DBA/2 [+Pol] ○
Figure 8 - Cell surface expression of TLR4/MD2 on peritoneal resident cells of BALB/c (Heavy line) and DBA/2 (Bold line) mice. PECs were blocked with 2.4G2 and stained with PE-conjugated TLR4. Expression of TLR4 shown in histograms [A]. Similar experiments were performed with thioglycollate elicited PECs and TLR4 expression is shown in [B]. Intracellular expression of TLR4 was examined on permeabilized PECs from both strains of mice. [C]
Fig. 12. Identification of filarial specific innate receptors on human phagocytes

Green line: Conjugate Control
Red line: Antigens binding to monocytes of filariasis infected cases
Blue shaded area: Antigens binding to monocytes of cases free of infection

Fig. 13

Binding of S. digitata antigens to human monocytes

Biotinylated soluble antigens from adult female worms were tested for binding to normal human monocytes and quantified by flow cytometry in the gated population; mean ± SD of 9 samples (P<0.0001)
Inhibition of binding of filarial antigen to monocytes

The specificity of binding of biotinylated filarial antigen to monocytes by competitive inhibition with cold non-biotinylated antigen at a ratio of 1:1 (n=7; P=0.0032)

Flow cytometry:
Binding of filarial antigen to lymphocytes in spleen of three different mice strains - (CBA/J, n=4; C3H He/N, n=6 and Balb/C, n=6). There was no statistically significant difference between the mice strains.
1.4 Post-DEC reactions in Human Bancroftian Filariasis: An Immunobiological study in Orissa, India

Objectives:

1. To study the role of endosymbionts *Wolbachia* in mediating reactions after administration of Diethylcarbamazine in infected human subjects.
2. To study the role endosymbionts *Wolbachia* in mediation of inflammatory responses in human filariasis during acute disease episodes.

Background:

Two different approaches were taken in the study as described under methods. In the first, cohorts of Mf carriers have been treated with Doxycycline or placebo and then treated with DEC to monitor overt clinical reactions as well as plasma cytokine levels to score inflammation. The working hypothesis is that if *Wolbachia* are responsible post DEC reactions, treatment with Doxycycline would effectively result in absence of reactions after DEC administration in the treated cohort. The study is being conducted in 3 phases, the first phase was completed fully in October 2004 and the second phase will be completed in June 2005. The third phase will be completed in October 2005 and the results will be compiled at the end of 3rd phase. The second approach is take three groups of subjects: 1) Asymptomatic Mf carriers 2) Subjects with cryptic infection, i.e., amicroilaraemic but with circulating filarial antigen and 3) patients with chronic disease but with no demonstrable infection and to treat all the three cohorts with single dose of DEC and monitor reactions.

Flow cytometry:

Binding of Filarial antigens to macrophages in spleen of three different mice strains (CBA/J, n=4; C3H He/N, n=6; Balb/C, n=6) are comparable.
and cytokine and Wolbachia levels and pre and post DEC administration. The observations made are shown in Table 1.

**Work Progress:**

The project addressed the issue of reactions observed in human communities after administration of Diethylcarbamazine citrate, the anti-filarial drug being currently used for control of lymphatic filariasis. It is generally believed to be associated with microfilarial density in the subject although empirical data for this is not available. Since post-DEC reactions often appear similar to LPS mediated inflammation and an endobacteria such as Wolbachia are known to reside in Mf, the current study was undertaken to investigate the association between Wolbachia density and post-DEC reactions. The underlying principle is that Wolbachia are susceptible to tetracyclines/doxycyclines and DEC mediated reactions should be preventable in Mf carriers by pre-treatment with the above antibiotics. Two strategies were followed; first, to treat Mf carriers with doxycycline for different duration and then administer DEC to monitor reactions both clinically as well as sub-clinically by measuring inflammatory molecules viz., TNF-a, IL-6 and RANTES as well as Wolbachia to analyze correlations between them; second, to treat cohorts of subjects, (with and without patent infection) with DEC and analyze correlations as described above. The first approach is being pursued independent of the second approach; it is being done in three phases- in each phase 4 groups of Mf carriers are being used, one placebo and three treated with doxycycline for different durations (5, 10 and 21 days) and subsequently treated with DEC to monitor reactions; the first phase and second phase have been completed and phase 3 is underway. The final data with analysis is expected to be available by the end of 2005. Treatment of mf for 21 days with doxycycline resulted in significant decrease of Wolbachia in mF during treatment administered for 10 to 5 days did not significantly decrease mf Wolbachia load and there was no change in placebo treated group (Fig. 1, 2 & 3). Post DEC reaction correlated significantly with higher level of Wolbachia in Mf indicating that Wolbachia are the possible origin of adverse reactions in Mf carriers (Fig.4). The following is the summary of results for the second approach: 1) Pre-treatment TNF-a levels were significantly more in Mf carriers (AS) and patients with chronic filarial disease (free of detectable infection) in comparison to subjects with cryptic infection (amicrofilaricmic with filarial antigenemia only), 2) Post–DEC reactions were significantly more in AS and CH cases as compared to CR cases and prevalence was comparable in the two (AS & CH) groups, 3) post DEC reactions were associated with significant elevation of TNF-a only in AS cases and not in CH cases, 4) conversely, significantly elevated levels of RANTES was observed only in CH cases and not in AS cases after administration of DEC, 5) plasma IL-6 levels were found to be significantly elevated in AS cases in comparison to CR and CH categories (pre drug administration) and after DEC administration, the levels of IL-6 decreased significantly in CR and CH cases and not in Mf carriers, and 6) plasma Wolbachia levels (as shown by real-time PCR) significantly decreased within 24 hrs after DEC consumption in CR and CH groups and not in the AS group.
The post DEC reactions in microfilaraemic subjects and patients with chronic disease were comparable. The reactions were negligible in subjects with cryptic infections. This suggests essentially that post-DEC reactions are not restricted to subjects with active current infection and that patients with chronic disease without demonstrable filarial infection also could display reactions. This notion is further confirmed by the lack of reaction in subjects with cryptic infection. Expectedly, Fig 1 and fig 2 show absence of relationship any significant relationship between Mf density (fig 1) and Wolbachia density (Fig 2) with post-DEC reactions.

### Table: Post-DEC Reactions in Various Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>PRE DEC Level</th>
<th>POST DEC Level</th>
<th>Clinical Reaction till 72 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RAN (ng/ml)</td>
<td>TNF-α (pg/ml)</td>
<td>IL-6 (pg/ml)</td>
</tr>
<tr>
<td>1. MF carrier (n=10)</td>
<td>168.5±56.91</td>
<td>73.76±22.73</td>
<td>1251±406</td>
</tr>
<tr>
<td>2. Cryptic infection (n=19)</td>
<td>185.6±38.63</td>
<td>38.94±8.188</td>
<td>475.3±106.4</td>
</tr>
<tr>
<td>3. Chronic disease (n=18)</td>
<td>115.2±22.33</td>
<td>79.91±30.29</td>
<td>532.3±130.6</td>
</tr>
</tbody>
</table>

*Post RANTES increased in significantly in comparison to pre levels (t=2.14, p=0.0409)

**48 hrs reaction is significantly more in AS group than Cry groups p=0.023.

***Post IL-6 decreased significantly in group CRY (t=2.488; p=0.0176) and CH (t=2.840; p=0.0080) groups.
There was a significant increase in plasma TNF-α levels at 24 hrs after administration of DEC in Mf carriers and not in CR and CH cases (Fig 5). Conversely, RANTES was found to be elevated only in CH cases and not in AS and CR subjects (Fig 6) indicating a dichotomy in the mechanism involved in post-DEC reactions observed in AS and CH cases.
1.5 Identification of serum immunosuppressive factors in human filariasis

Objectives:
1. To identify the immunosuppressive factors in sera of microfilaraemic subjects.
2. To correlate the degree of immunosuppression with presence/intensity of infection with adult stage parasite.

Initial investigation indicated that microfilaraemic sera mediated profound inhibition of PHA induced T-cell proliferation. An attempt has been made to characterize the immunosuppressive factors in microfilaraemic sera. Inhibitory serum factor was dialyzable. The serum inhibitory factor was found to be resistant to treatment at 56°C for 30 mins. Further, Aminoguanidine, an inos inhibitor failed to reverse serum mediated inhibition. The possible cytotoxicity to lymphocytes mediated by inhibitory sera was studied. Identification of apoptotic cells in 96hrs cultures was performed by flow cytometry using annexin V – PE apoptosis detection kits. Serum inhibitory factor(s) in mf carriers induced apoptosis of lymphocytes as shown by Annexin V staining (Fig-1). Analysis of factors and the mechanism of induction of apoptosis are currently under study.

Indirect evidence indicates that IL-10 and/or TGF-β play a role in generating hyporesponsiveness to parasite antigens. Thus both IL-10 and TGF-β have been shown to play an important role in down regulating antigen specific proliferative responses in microfilaraemic subjects. However the relationship between IL-10 and TGF-β with immunosuppression observed in human filariasis is still not known. The relationship between IL-10 and TGF-β with the degree of immunosuppression is being analyzed. TGF-β levels has been quantified in inhibitory microfilariaemic sera and correlated with % inhibition. TGF-β levels in sera were found to correlate inversely with % inhibition (Fig-2). We had demonstrated earlier that significantly elevated levels of IL-10 in acute filariasis in comparison to endemic controls, Mf positive cases and cryptic cases. IL-10 levels in culture supernatants of PHA stimulated Peripheral Blood Mononuclear Cells (PBMC) in presence or absence of inhibitory sera did not correlate with % inhibition.

Fig. 1.

INDUCTION OF APOPTOSIS BY INHIBITORY SERA: HISTOGRAM WITH STATISTICS FOR ANNEXIN V STAINING BY FLOW CYTOMETRY

CONTROL WITH 10% AUTOSERA

CPM-308

PHA WITH 10% AUTOSERA

CPM-27,315
Fig. 2.

**On Going Studies**

**PHA WITH 10% INHIBITORY SERA**
(BP-89)
CPM - 1,604

**PHA WITH 10% INHIBITORY SERA**
(BP-143)
CPM - 469

**OVERLAYERED HISTOGRAM**

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**TGF beta (pg/ml)**

<table>
<thead>
<tr>
<th>% inhibition</th>
<th>TGF beta (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>75,000</td>
</tr>
<tr>
<td>10</td>
<td>50,000</td>
</tr>
<tr>
<td>20</td>
<td>25,000</td>
</tr>
<tr>
<td>30</td>
<td>0</td>
</tr>
</tbody>
</table>

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1.6 Development and evaluation of community development and partnership strategies for drug delivery for the control of lymphatic filariasis in urban areas of Orissa, India

Background:

Lymphatic filariasis (LF) is a major health problem in many countries of the tropical world. However, recent advances in mapping, diagnostics and development of chemotherapy and monitoring tools have made it possible to plan for the elimination of the disease. The World Health Assembly in 1997 passed a resolution for the global elimination of lymphatic filariasis by the year 2020. India, in its national health policy committed to eliminate LF by the year 2015. Most practical and feasible method of controlling LF is rapid reduction of microfilarial load in the community by annual mass drug administration (MDA) of single dose of diethylcarbamazine (DEC) alone or combination of DEC and albendazole, and it has already been initiated in India. Recently completed TDR sponsored multi-centric study showed coverages to be far below the expected levels at all study sites. This study was conducted primarily in rural areas and highlighted the need for advocacy and development of better delivery strategies for achieving high levels of coverage. Also the results from Orissa indicated poor compliance in urban areas. Urban areas (vs. rural areas) recorded lower coverage (45% vs. 76%) and compliance rates (23% vs. 49%).

While a drug delivery strategy for rural areas has been developed and is being continuously modified no such strategy exists for drug delivery in urban areas. Bancroftian filariasis is recognized as a disease of urbanization and there is a growing need to develop strategies for drug delivery to achieve high levels of compliance in urban areas. Urban populations also differ from rural populations in several ways. In addition the problem of migration is more pronounced in urban areas than in the rural areas. The higher levels of literacy and economy make these populations more demanding in terms of information and quality of services. Similarly the affluence of some urban communities makes them rely heavily on the private sector for the health needs. More importantly the primary health care system in urban communities lacks the infrastructure and the outreach that is found in rural areas. Thus urban drug delivery strategies, which take into consideration these factors, need to be developed well in time before the mass drug administration strategy is expanded to cover more districts and urban areas including large metropolitan cities.

Hence, the present study has been initiated to develop an innovative strategy to achieve higher coverage of MDA in urban areas. As Phase-1, a formative research has been undertaken to explore and identify opportunities in urban communities, which would help design innovative urban-specific intervention strategy for MDA for elimination of LF. This study has been undertaken in a small industrial town in Orissa, India with a population of around 52000. Various qualitative and quantitative research methods are employed during formative research. The results of formative phase indicated conduciveness for intervention with more community participation and partnership approach. The study also attempted to explore the opportunity for linkages and potential for community strategies with regard to MDA. Stakeholders like community leaders, private practitioners, non-governmental organisations, community-based organisations like women groups and youth groups expressed willingness to participate in activities related to MDA. The data also indicated the need to develop some sub-group specific approaches to achieve higher coverage in MDA. Thus the results of formative research helped to develop a strategy with community participation and partnership approach specific to the urban communities, to achieve higher coverage of MDA. The results of formative research are presented in previous annual report (RMRC Annual Report, 2003-04).

Research questions for intervention:

1. To what extent are communities and potential partners in urban areas aware of filariasis and motivated to participate in MDA?
2. To what extent can the low treatment coverage in urban areas be improved by the application of a strategy that involves stakeholders, especially active CBOs/NGOs and private practitioners, as equal partners in planning, decision-making and implementation?

3. Can such a strategy significantly enhance the perceived need of, and support for LF treatment among the community, the health workers and the municipality officials?

4. Is effective application of the alternative strategy possible using the existing human resources at the municipality and community level, and if not, what else is required?

5. What level of treatment compliance can be achieved through the application of such an alternative strategy?

6. Is the strategy cost-effective for achieving the required coverage in urban areas (taking also into account the contributions by different partners, including the community)?

**Purpose and objectives of the intervention:**

**Purpose:**

To develop and test the partnership strategy for mass drug administration, which would achieve the desired high treatment coverage in urban populations necessary for elimination of LF.

**Objectives:**

1. To test an intervention strategy that addresses the challenges for MDA in urban areas, building on an inclusive partnership framework developed on the basis of the research findings on the above objective, and involving in particular the private practitioners and active CBOs.

2. To evaluate the impact of this intervention strategy on perceived need of, and in enhancing support for, MDA amongst all stakeholders including the community, health workers, municipal officials.

3. To describe the preparatory and mobilization process as developed by the stakeholders, and to assess its strengths and weaknesses.

4. To describe the drug distribution process as developed by the government in consultation with the stakeholders, and to assess its strengths and weaknesses.

5. To evaluate the treatment coverage (consumption rate) achieved with the new strategy, and to assess whether after three years of intervention it reaches the desired level of treatment coverage with DEC/Alb that is required for elimination of LF.

6. To determine the feasibility of implementation of the new strategy using existing human resources (health and other sectors) at the municipal and community level.

7. To document the contributions made by various stakeholders and to determine the cost of the new strategy.

**Study design:**

The intervention has been initiated with community participation and partnership strategy to implement MDA. During this phase the researchers acted as facilitators. The existing health system along with municipality and other partners implemented the intervention. The details of intervention and MDA are described in this report. The evaluation is carried out by the researchers. The coverage survey is carried out in a non-intervention urban area and rural area along with intervention urban area.

**Study areas:**

The intervention is carried out in an urban area, Choudwar. The formative research has been carried out in this area. Choudwar is a municipality area in Cuttack district. Cuttack
is one of the coastal districts of Orissa, which is endemic for LF. Choudwar is situated on the north bank of Birupa River, a branch of river Mahanadi. Choudwar is an industrial area having six major industries and several small-scale industries. However, majority of them have been declared as sick industries and therefore remain closed. As per the Census of India (2001), the population of Choudwar town is 52,498 of which 28,243 (53.8%) are male and 24,255 (46.2%) are female. Of the total population around 55% are workers, which include 28.3% of specifically industrial workers. The health infrastructure of Choudwar is far from satisfactory. Though there is a municipal hospital, the posts of medical officers and many other paramedical have been lying vacant for a long time. The health needs of the industrial workers are being catered by an ESI hospital and an ESI dispensary. Hence, majority of Choudwar’s general population depend on private practitioners and hospitals. An NFCP unit exists in Choudwar to undertake antilarval activities and filarial surveys. However, the unit does not function well due to lack of infrastructure and manpower. The people of Choudwar have not been exposed first time to mass drug administration, as Cuttack district did not come under the control programme till then. Choudwar consists of civic body, i.e. municipality to look after the administration of urban communities. The sanitation and public health also come under the purview of municipality. The entire urban area is divided in to 17 wards. The municipality has a constitutionally elected body consisting of a chairman and members. Each ward is represented by a member, i.e. councillor and he is elected democratically from the adult members of the ward.

Various Processes of Proposed Intervention:

Formative research conducted in Phase-1 indicated a conducive atmosphere in the study area for the implementation of mass drug administration (MDA) of diethylcarbamazine (DEC) with the approaches of partnership and community participation. Many health programmes viz., pulse polio programme, leprosy eradication programme, hepatitis control programme, blood donation programmes, AIDS awareness campaigns, etc. are organised in the study area with the help of local communities. Health camps are organised every year by the municipality as well as youth clubs in which people’s participation is quite satisfactory. Besides health programmes, some community based organisations (CBOs) have also organised many cultural programmes with the active involvement and participation of people. People of all groups extended their cooperation generously and also there are no major conflicts during community related activities. Implementation of MDA is a government driven programme and municipality is the local government responsible for public health. Though municipality has no such health infrastructure, time-to-time it has conducted many health programmes efficiently with the assistance of local CBOs. Besides during formative research many key informants including doctors, media personnel, members of CBOs, NGOs and local leaders opined that municipality should carry out MDA. The key informants also opined that municipality can provide all logistical support like it has a big town hall with well sitting arrangements and sound system for public propagation, etc. It has also a Filaria unit, which is not functional, but supportive staff is there. Although a well-equipped municipality hospital is not present, it has a dispensary and a pharmacist working there. Taking all these factors into consideration, it was felt that the municipality can play a key role in undertaking MDA in the study area.

Stakeholders’ active involvement in planning and decision-making: In this programme, municipality and local health institution played key roles. This body succeeded to include many stakeholders like bureaucrats of municipality, private practitioners, practitioners from other governmental and non-governmental hospitals, community based organizations (CBOs) like youth clubs, women clubs and residence associations, non-governmental organizations (NGOs), journalists, representatives of industries, prison and schools, and representatives of religious and ethnic groups in the program. This newly formed group named as steering committee met periodically. The research team initially advocated for the program to different stakeholders by briefing rationale and benefits of
the program. As facilitators the research team also shared the results of formative research with the steering committee. The steering committee took all decisions on planning including social mobilization and drug distribution. Nominal group technique was used to arrive at a consensus whenever required. However, the committee received some directions and suggestions from the local health institution, which was responsible for providing drugs and other materials.

**Advocacy:** Advocacy was done both among the populations and key partners. This task was performed by the research team for the steering committee and the latter did the advocacy among the local groups. Steps were also taken for the inclusion of new zealous partners and motivating further the existing stakeholders. The steering committee undertook this activity among the community groups.

**Initiating the intervention:** Having designed the plan for the entire urban area, the steering committee identified ward level partners, who are suitable to undertake activities related to community mobilization and drug distribution. The strengths and weaknesses of these groups were assessed by the steering committee and necessary inputs were given. In most of the wards (15 out of 17 wards), the concerned councillors led the ward level activities. In the remaining two, a social worker and a former councillor did the same. The ward level groups met before the MDA and made suitable plans for their ward, through an intensive profiling process.

**Partnership:** Thus the partners were involved in various stages of intervention. Also some of the stakeholders got involved in the social and economic mobilization. The micro-level planning (methods of MDA, date, time, duration, selection and training of community drug distributors, etc.) at the municipality and ward level were made by the steering committee and the ward level committee, respectively. A few medical practitioners from the steering committee were grouped into four teams for adverse reactions surveillance and management as an integral part of the distribution process. The basic objective was to minimize the damage expected during drug distribution and enhance people's confidence on the program. In addition, different sub-groups were identified for the separate differential treatment (Box-1).

**MDA:** The strategy ultimately resulted in local decision-making in consultation with health institution with regard to execution of drug distribution. The following were the major components in MDA.

- **Selection and training of community drug distributors (CDDs):** The ward level committee identified some local volunteers, designated as CDDs, to distribute the drugs. Each CDD was given charge of a geographical area consisting of population around 400. Some para-medical staff of the local health institutions were included as supervisors to monitor the MDA process. The CDDs and supervisors underwent one-day training, organized by local health institution with the help of the steering committee.

- **Drug distribution:** The distribution process initiated on the morning of September 15, 2004, as planned jointly by the steering committee and ward level committee. The distribution was carried out by the CDDs either individually or in groups. In some wards, the members of steering committee, particularly the councillors and members of ward level committees, monitored the activities. The supervisors monitored the activity of drug distribution and assisted the adverse reactions management team by identifying cases along with the CDDs. The distribution process continued subsequently for three more days, but adverse reactions management teams did not conduct mobile operation during those days due to practical exigencies. However, the CDDs, supervisors and ward level committees were directed to bring such cases to respective hospitals of these physicians.