Responses of *Brugia malayi* – Indian leaf monkey (*Presbytis entellus*), a non-human primate model of filariasis, to diethylcarbamazine, ivermectin and CDRI compound 82-437

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We have earlier developed the Indian leaf monkey (*Presbytis entellus*) – *Brugia malayi* model of human filariasis that develops the characteristic filarial disease manifestations such as episodic febrile attacks and limb oedema. We report here the responses of the model to known antifilarials, diethylcarbamazine (DEC) and ivermectin (IVM) and a new antifilarial CDRI compound 82-437. DEC treatment at 18 and 200 mg/kg × 5 days induced respectively, 53.4 and 86.1% reduction in microfilaraemia by day-8 post-treatment (p.t.). Microfilaraemia further decreased by 90–99% between day 14 and day 63 p.t. IVM (1.5 mg/kg × 5 days) induced 45 and 90% fall in microfilaraemia by day 7 and day 21 p.t. respectively. Compound 82-437-treated animals showed gradual decrease in microfilaraemia after day 7 p.t., with more than 95% suppression on the day of autopsy. Untreated infected monkeys showed characteristic increases in microfilaraemia till day 63 p.t. In both DEC and compound 82-437-treated animals, microfilaraemia was associated with changes in eosinophil count and immune responses. The adult worm recovery from lymph nodes/lymphatics was 0, 4.6, 1.1, and 5.3% in DEC-, IVM- and compound 82-437-treated and untreated monkeys, respectively. Compound 82-437-treated monkeys showed enlarged popliteal and inguinal lymph nodes with a few intact worms and granulomas. No lesions were detected in the nodes of DEC-treated monkeys. Nodes in untreated controls showed granulomas, follicular atrophy, sinus histiocytosis, and intact or phagocytosed mf in sinuses. Adult worms were found in the afferent lymphatics with or without associated lymphangitis.

It is concluded that the Indian leaf monkey – *B. malayi* model responds to antifilarial treatment in a predictable manner, with the parasitological profile and changes in absolute eosinophil count (AEC), immunological and histological parameters largely resembling those of human subjects.

THE sub-periodic strain of *Brugia malayi* has been found to be easily transmittable to the Indian leaf monkey (*Presbytis entellus*). The model also mimics some of the char-

acteristics of human filarial manifestations such as fever, episodic attacks of limb oedema[1,2], persistent limb oedema or scrotal swelling and other pathophysiological responses[3,4]. However, the utility of such a non-human primate model for tertiary screening of potential filaricides depends on the similarity of the host–parasite relationship and its response to known filaricides. The present study was therefore undertaken to evaluate the chemotherapeutic response of known antifilarials, diethylcarbamazine (DEC), ivermectin (IVM), standard drugs for filariasis, and compound 82-437, an orally active adulticidal antifilarial developed at the Central Drug Research Institute (CDRI), in *B. malayi* – rodent model[5]. Further, since the efficacy of antifilarials like DEC and IVM has been linked to their ability to alter the immune responses of the host[6–8], certain immunological parameters such as circulating immune complexes, filaria-specific IgG, lymphocyte proliferative responses to filarial antigen and mitogen were also investigated along with the assessment of changes in the lymph nodes and other lymphoid organs following treatment with DEC, IVM and CDRI compound 82-437. The present article reports the results of such a study.

Materials and methods

Animals

Young adult male Indian leaf monkeys, commonly known in Hindi as langur, of 3–4 kg body weight were obtained from local suppliers. The animals were immediately kept in quarantine for 45 days during which they were subjected to routine health-check procedures, including clinical biochemistry and hematology and were thoroughly examined for tuberculosis (TB; using Mantoux test and chest X-ray), intestinal helminthiasis (examination of faeces), and microfilariae (by night-blood examination). Animals found positive for intestinal helminths were treated with mebendazole (Zodex, Concept Pharmaceuticals, Mumbai, India) at 20 mg/kg, p.o. for 3 days, which was repeated after three weeks. None of the monkeys was positive for filaria. On completion of the quarantine and health-check, the animals were transferred to animal quar-

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ters of the experimental filariasis wing where they remained under observation for not less than four weeks before the start of the study. Two days before the start of the study, the animals were again subjected to all the above tests, except those for TB, for a final health-check. Disease-free monkeys, negative in all the above tests, were finally selected for exposure to filarial infection. Throughout the pre-study and study period the animals were housed at temperature- (24–28°C) and photoperiod- (12 h-dark and 12 h-light) controlled quarters protected from mosquitoes and other vectors by screening wire. The animals were fed on a commercial pellet diet (Nav Maharashtra Chakan and Oil Mills, Pune, India) supplemented with calculated quantities of bread, Bengal gram, seasonal fruits and vegetables. They had free access to safe drinking water.

Infection

Infective third-stage larvae (L_3) of B. malayi were obtained from laboratory-bred female Aedes aegypti mosquitoes fed on microfilaraemic Mastomys coucha as described previously[9]. Each monkey was given a total of 500 L_3/animal in 4–5 inoculations in insect saline within a span of 20 days. All the inoculations were given subcutaneously in the ankle of one leg only. Density of microfilaraemia (between 9 and 10 p.m.) of infected animals was measured by membrane filtration technique[1] on days 70, 80 and 90 post-first L_3 inoculation (p.i.). Microfilariae-positive monkeys were included in this study.

Determination of total leukocyte and absolute eosinophil counts

Total leucocyte count (TLC) and absolute eosinophil count (AEC) were made by standard method using Naubauer counting chamber, as described by Dacie and Lewis[10]. Briefly, 5 and 20 μl of tail blood from each animal was taken and diluted (20 times) in Turks fluid or eosinophil diluting fluid respectively, and kept for 5 min. The counting chamber was then charged with 10 μl of the diluted blood and cells were counted under the microscope.

Treatment

Fifteen microfilaraemic animals harbouring 80–90-day-old infection were randomized to five groups (I–V), each consisting of three animals. Groups I and II were subjected to DEC-citrate (18 and 200 mg/kg × 5 days respectively), while animals of groups III and IV were treated with IVM and compound 82-437 at 1.5 mg/kg and 200 mg/kg respectively, for five days. The antifilarial agents were administered by stomach tube. Group V, consisting of three infected animals, was treated with an equivalent

volume of the drug diluent, distilled water and served as control.

Parameters

Microfilaraemia density, total and differential leucocyte counts, filaria-specific IgG, circulating immune complexes (CICs) in blood, lymphocyte proliferative response to filarial antigen or mitogen and rectal temperature were carried out before treatment and on days 10/14, 35 and 60–65 p.t. Histopathology of lymph nodes and associated vessels from treated and untreated animals was carried out on day 60 p.t. only.

Gross pathology

All the treated and untreated animals were observed daily for side reactions, if any. Rectal temperature was also measured daily before and after treatment.

Parasitological

All the animals were autopsied between days 60 and 65 of treatment. Before autopsy, animals were inoculated with 5 ml 1% Evans blue into the dorsal side of the feet of both fore and hind limbs to visualize the lymphatics. After 30 min, the animals were sacrificed with an overdose of Pentothal sodium. Worms were recovered from lymphatics following the method of Buckley and Edeson[11]. Lymph nodes of all sites along with their afferent and efferent lymphatic vessels were taken for histological studies. Only the above parasitological parameters were studied in animals treated with IVM. Mean adult worm recovery was expressed as the percentage of the infective inoculum.

Immunological

Preparation of antigen: The antigen used was soluble somatic extract of B. malayi adult worms. It was prepared as described by Tandon et al.[12] with some modifications. Briefly, the worms were collected from the peritoneal cavity of experimentally infected jirds (Meriones unguiculatus)[13] and were homogenized with 0.01 M phosphate buffered saline (PBS; pH 7.2), in a Porter Elvejhm tissue grinder (A. Thomas Scientific, Philadelphia, PA) at 4°C. The homogenate was sonicated on ice at 20 Kc/s for 20 s. After 10 such strokes the extract was centrifuged at 100,000 g for 1 h and filtered through a 0.22 μm syringe filter (Millipore, Bangalore, India). The protein content was measured by the method of Lowry et al.[14] and the antigen was stored in aliquots of a 0.2 ml volume at – 20°C until used.

Isolation of peripheral blood mononuclear cells: Peripheral blood mononuclear cells were isolated from heparinized venous blood of the animals by Histopaque (Sigma Chemicals, St Louis, Missouri) gradient centrifugation[15]. Cell viability was checked by the Trypan blue dye exclusion method. Viability of cells was greater than 95%.

Estimation of B- and T-cells: B-cells were estimated according to the method of Bianco *et al.*[16]. Briefly, sheep erythrocytes (SRBCs) were washed in veronal buffer (VB, pH 8.6). An equal volume of 5% SRBC in VB and 1/1000 dilution of hemolysin was incubated at 37°C for 5 min. The SRBCs coated with anti-sheep SRBC antibody, i.e. erythrocyte amboceptor (EA) were washed thrice and incubated with normal monkey serum (optimum concentration) for complement in equal volume at 37°C for 30 min. After incubation, cells were washed and a final concentration of 0.5% EA cell suspension was mixed with lymphocytes $(3 \times 10^6/\text{ml})$ for 30 min followed by incubation overnight at 4°C. Erythrocite coated with antibody and complement (EAC)-rosettes, i.e. B-cell rosettes were counted in a haemocytometer and expressed as a percentage of total lymphocytes. Lymphocytes bearing three or more erythrocytes on their surface were taken as positive rosettes.

For T-cell estimation, the method of Bloom[17] was broadly followed. Lymphocyte cell suspension, as described for estimation of B-cells, containing $4 \times 10^6/\text{ml}$ lymphocytes was made in Hank's Balanced Salt Solution and a 0.5% SRBC suspension was also made. An equal volume (0.5 ml) of lymphocyte suspension and 0.5% of SRBC was mixed in duplicate in round-bottomed tubes. It was centrifuged at 75 g for 10 min at room temperature and later maintained at 4°C for 40 min and then gently resuspended. Then 2% glutareldehyde in normal saline was added to this to get a final concentration of 0.66%. The tubes were then centrifuged for 15 min at 75 g and kept at 4°C for 15 min. They were then suspended and the rosettes were counted in a haemocytometer. Lymphocytes bearing three or more erythrocytes on their surface were taken as positive rosettes.

Antibody determination by enzyme linked immunosorbent assay (ELISA): Filaria-specific IgG was determined by ELISA, broadly following the method of Voller *et al.*[18] with some modifications[19]. Briefly, ELISA plates (Nunc, Denmark) coated with 1 μg/ml of the antigen in 0.06 M carbonate buffer (pH 9.6); animal sera (primary antibody) were used at 1 : 500 dilution and rabbit anti monkey IgG-peroxidase conjugate (Sigma Chem. Co., St. Louis) was used at 1 : 2000 dilution. Absorbance was read at 492 nm in an ELISA reader (Multiscan). The semi-quantitative levels of IgG were expressed as OD values.

Determination of CICs: Broadly, CICs in the sera of monkeys were determined by ELISA according to Matsumura *et al.*[20], with some modifications[3] using anti-human Clq (Sigma Chemical Co, St Louis). Microtitre wells (Nunc, Denmark) were sensitized with 100 μl of anti-human Clq (1 μg/ml) in PBS. Sera were used at 1 : 500 dilution and rabbit anti-monkey IgG-peroxidase conjugate was used at 1 : 2500 dilution. The procedure for ELISA was the same as followed for IgG determination.

Lymphocyte transformation test: The assay was performed as described by Ghosh *et al.*[4]. Peripheral blood mononuclear cells (PBMCs) at the concentration of $2 \times 10^5/\text{well}$ in 0.2 ml in triplicate were incubated in flat-bottomed 96-well culture plates (Nunc, Roskilde, Denmark) with *B. malayi* adult worm antigen (10 μg/ml) and Con A (5 μg/ml) in RPMI-1640 medium, supplemented with 10% foetal calf serum, penicillin (100 U/ml) and streptomycin (100 μg/ml) for 96 h (Con A) and 150 h (antigen) at 37°C under 5% CO_2 atmosphere. Control cultures (medium and cells only) were also run simultaneously. The cells were then pulse-labelled with [^{3}H]-thymidine (1 μCi/well) and harvested after 18 h. [^{3}H]-thymidine incorporation was counted in a liquid scintillation β-counter (1217-Reckbeta, Finland). The results were expressed as a stimulation index (SI), calculated by dividing mean counts per minute of stimulated culture with mean counts per minute of unstimulated culture. SI more than two was considered responsive.

Histopathological

Lymphnodes and their vessels of both control-untreated and treated animals were subjected to buffer formaldehyde fixation followed by histopathologial staining of 4–5 μm thick sections of tissues and using routine hematoxylin–eosin staining. Pathological alterations in tissues were observed under a microscope.

Results

Effect on microfilaraemia and adult worms

Microfilaraemia in infected untreated animals was elevated throughout the observation period of 63 days (Figure 1 *a*).

On autopsy, untreated control animals showed an adult worm recovery of about 5.3% of the infective larvae inoculated, and the female worms were gravid. Figure 1 *b* depicts the effect of DEC, IVM and compound 82-437 on microfilaraemia of animals with *B. malayi*. DEC exerted dose-dependent microfilaricidal efficacy. On day 7 p.t., the lower dose (18 mg/kg) exerted 53.4%, while the 200 mg/kg dose produced more than 80% lethal effect on mf. Thereafter, at both dose-levels, a gradual fall in mf count was observed with 90–99% suppression in microfilaraemia till the day of sacrifice (63 p.t.).

On autopsy, none of the DEC-treated animals had worms in the lymphatics or lymph nodes.

IVM exhibited a gradual decrease in microfilaraemia from day 7 (45%) to day 21 (90%). After day 21, the mf count remained low till the sacrifice of the animals. Autopsy revealed 4.6% recovery of adult worms from inguinal and popliteal nodes and their channels.

All the female worms contained degenerated and distorted mf in the uterine contents.

In compound 82-437-treated animals, the density of microfilaraemia on day 7 p.t. increased but thereafter it gradually decreased, with more than 95% suppression till the day of autopsy.

On autopsy, only a few worms (1.1%) could be recovered from the lymphatics and lymph nodes and the female parasites were 100% sterile.

Effect on various parameters

Immediately after treatment, whereas the higher dose of DEC (200 mg/kg) brought down the level of AEC significantly and remained low thereafter, the lower dose (18 mg/kg) showed increase in the level of AEC initially, but thereafter decreased to the pretreatment level. In untreated animals, the high eosinophilia correlated with the increasing microfilaraemia (Figure 2 *a*). Treatment did not affect TLC (Figure 2 *b*).
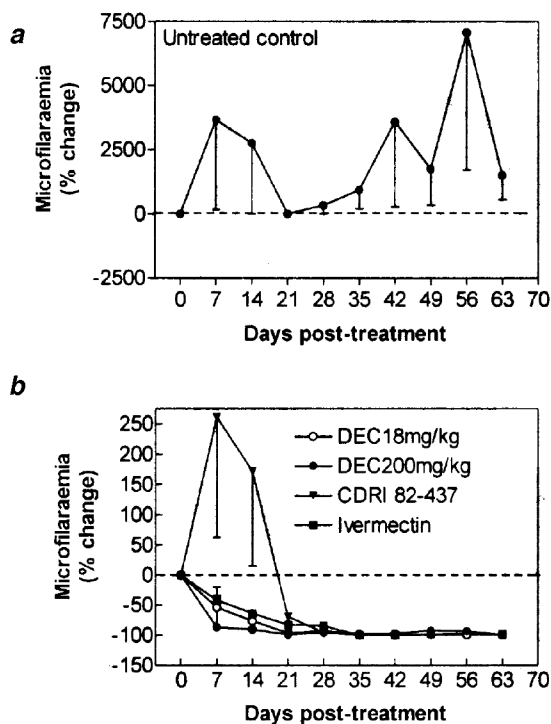
Figure 1. Microfilaraemia in (*a*) untreated and (*b*) diethylcarbamazine (18 and 200 mg/kg), ivermectin (1.5 mg/kg) and compound 82-437 (200 mg/kg)-treated *Brugia malayi*-infected Indian leaf monkeys. Values are mean ± SD of data of three animals.

Both treated and untreated animals showed an increase in B-cell count on day 7 or day 14 p.t. but it decreased significantly thereafter. However, on day 63 p.t., the level of B-cell count was significantly high in control animals compared to treated animals (Figure 2 *c*). T-cell count in untreated, DEC at 200 mg/kg, and compound 82-437
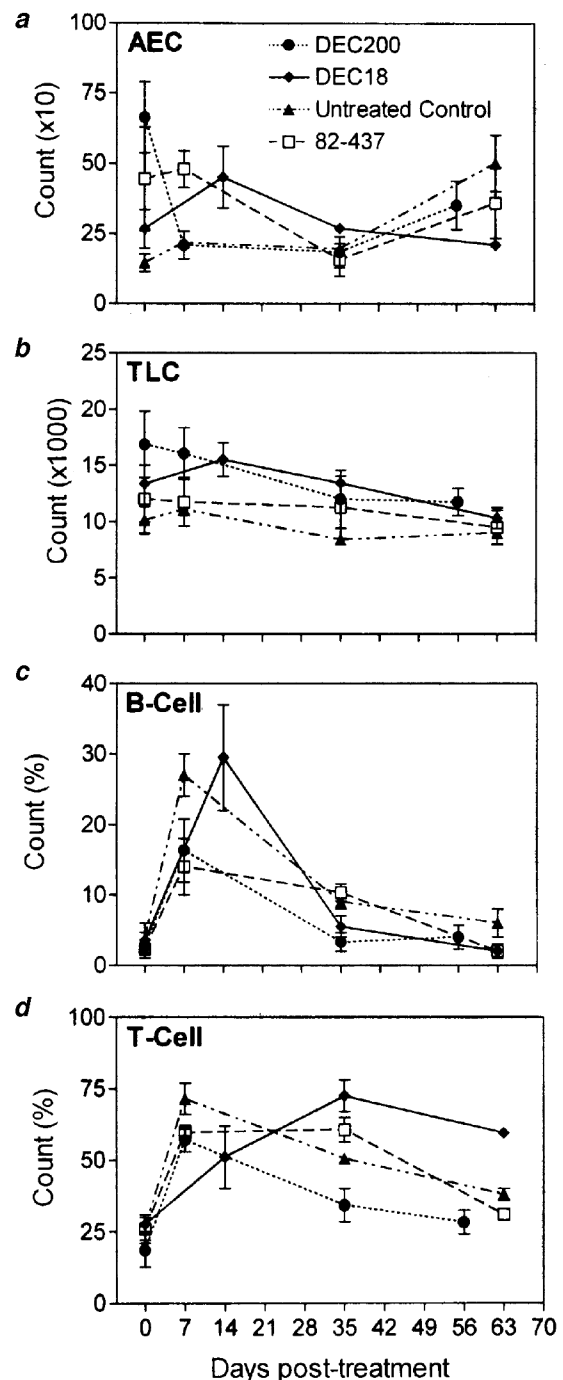
Figure 2. Absolute eosinophil (*a*), total leucocyte (*b*), B-cell (*c*) and T-cell (*d*) counts at different time periods after treatment of *B. malayi*-infected Indian leaf monkeys with diethylcarbamazine (18 and 200 mg/kg) and compound 82-437 (200 mg/kg). Values are mean ± SD of data of three animals.

increased immediately after treatment followed by a gradual decrease. However, treatment with DEC at lower dose caused increase in T-cell count on day 35 p.t. (Figure 2 *d*).

While the lower dose of DEC increased the PBMC proliferative response (CMI) to Con A, the higher dose of DEC, compond 82-437 and untreated controls showed unresponsiveness at all time points (Figure 3 *b*). Antigen-specific proliferation was unresponsive after DEC-treatment (both at 18 and 200 mg/kg) and in untreated animals (except at one time point, i.e. on day 10 p.t.), but in compound 82-437-treated animals, the filaria-specific proliferative response of PBMC gradually increased till the day of sacrifice (Figure 3 *a*). In contrast to low dose of DEC, the higher dose brought down the filaria-specific IgG levels immediately after treatment. IgG levels in compound 82-437-treated and untreated animals remained unaltered at all time points (Figure 3 *c*). In animals treated with higher dose of DEC or compound 82-437, the levels of CICs decreased after treatment but returned to pretreatment values in DEC-treated animals only. On the contrary, at lower dose of DEC the level of CICs increased after treatment. Untreated animals showed no alteration in CIC levels throughout the period of study (Figure 3 *d*).

Gross pathology and histology of popliteal and inguinal lymph nodes

One of the three animals treated with the higher dose (200 mg/kg) of DEC showed mild pyrexia (+ 0.5°F of normal temperature) from day 4 to 8 p.t. Gross pathology of all the DEC-treated animals showed palpable inguinal and popliteal lymph nodes immediately after treatment (between day 10 and day 15 p.t.). At the late phase of the observation period, none of the DEC-treated animals had shown palpable lymph nodes, nor was any worm or lesion found in the lymphatics, lymph nodes or tissues.

Macroscopic examination of untreated, IVM or compound 82-437-treated animals showed enlarged inguinal and popliteal nodes.

Microscopic examination of the tissues of animals treated with the compound 82-437-revealed a few intact worms (1.1%) and filarial granulomas with several multinucleated giant cells around an adult worm (Figure 4 *b*).

In untreated animals, microscopic examination of the popliteal and inguinal lymph nodes revealed sinus histiocytosis, granulomas and follicular atrophy along with intact, degenerating or phagocytosed mf in sinuses of lymph nodes (Figure 4 *a*). Adult worms were found in the afferent lymphatics of the nodes and, in one case this was associated with lymphangitis.

Discussion

The *Brugia* spp.–rodent model has been used extensively for primary screening and *B. pahangi* infections in dogs

and cats for secondary screening of potential filaricides. We have earlier developed a non-human primate model of filariasis by experimentally infecting the Indian leaf monkey, *P. entellus* with the human filaria, *B. malayi* and showed that this model develops the characteristic filarial
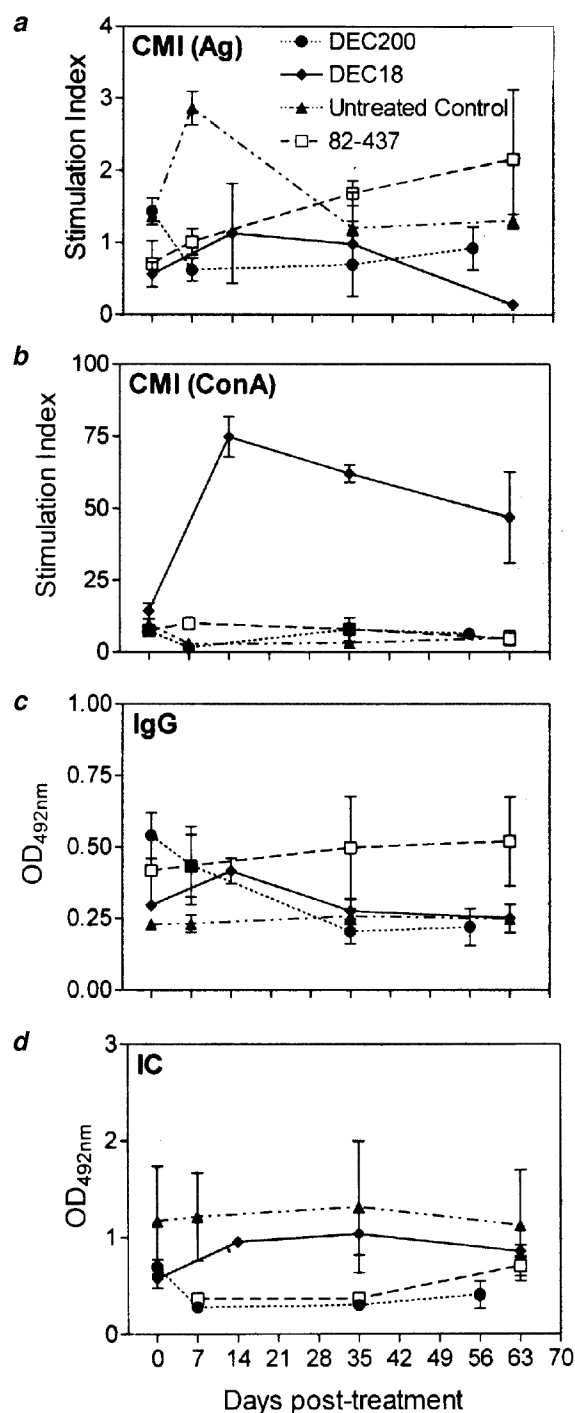
Figure 3. Cellular immune responses and IgG and immune complexes in *B. malayi*-infected Indian leaf monkey. T-cell proliferative response to Con A (*a*) and filarial antigen (*b*) and levels of filaria specific IgG (*c*) and ICs (*d*) at different time periods after treatment with diethylcarbamazine (18 and 200 mg/kg) and compound 82-437 (200 mg/kg). Values are mean ± SD of data of three animals.

disease manifestations, such as episodic febrile attacks and limb oedema[1,2]. We decided to investigate its potential for chemotherapeutic response of antifilarial agents
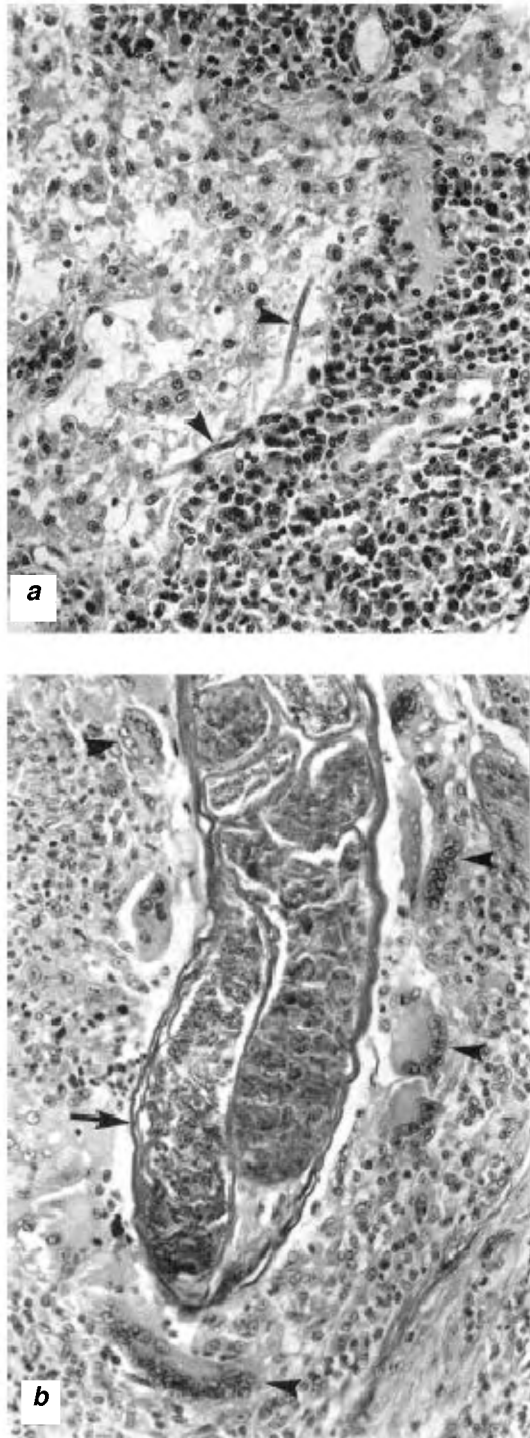
Figure 4. Popliteal lymph nodes from *B. malayi*-infected Indian leaf monkey. *a*, Node from untreated control monkey showing intact microfilariae (arrow heads) and several histiocytes in the cortical sinus. HE, × 128. *b*, Afferent lymphatic near the entry into the node from compound 82-437 treated monkey showing granuloma formation with several multinucleated giant cells (arrow heads) around an adult worm (arrow). HE, × 128.

in addition to monitoring the alterations in host–parasite relationship following antifilarial treatment. DEC, IVM and compound 82-437 have shown antifilarial efficacy in the present model, which was almost similar to that observed in rodent models[5,21,22]. An interesting finding was that pyrexia developed due to DEC (200 mg/kg) treatment in one of the three monkeys and this is in agreement with what is observed in microfilaraemic (*Wuchereria bancrofti*) humans following treatment with DEC. The other major observation of the present study was that IVM cleared circulating mf of *B. malayi* rather slowly in comparison to DEC. However, unlike DEC treatment, adverse reaction did not occur in any of the IVM-treated animals in spite of almost complete clearance of mf from the circulation. The lack of reactogenicity due to IVM treatment in the host might be due to slow action on peripheral microfilaraemia. However, *Acanthocheilonema viteae* in *M. coucha* revealed that in spite of 100% clearance of mf within 24 h with IVM, no animal died due to reaction, whereas in the same model when treated with DEC some animals expired within 24 h with lesser microfilaricidal efficacy. Borehem and Atwell[23] also reported lack of adverse reaction in *Dirofilaria immitis*-infected dogs after treatment with IVM.

In helminth infestations, the pattern of eosinophilia was found to be related to the level of microfilaraemia and the destruction of microfilaraemia in the spleen. However, in treated animals (DEC or compound 82-437), the level of AEC decreased with decreasing level of microfilaraemia. In filariasis, eosinophilia is known to be involved in antibody-dependent cellular adherence and killing of *B. malayi*-infective larvae in the presence of sera from patients with tropical pulmonary eosinophilia and elephantiasis with or without microfilaraemia[24]. A similar mechanism, which is involved in the killing of mf and the role of toxic cationic proteins, such as major basic proteins and eosinophil cationic protein, as well as oxidative and other enzyme systems involved in such parasite killing, has been reviewed[25]. We have observed that in high microfilaraemic monkeys there were huge granulomas in the spleen, with microfilarial fragments engulfed by foreign body giant cells and surrounded with eosinophils (unpublished). Based on these observations, we believe that the high eosinophilia observed in leaf monkey in the present study is associated with the destruction of mf in the spleen and other areas. In the present study untreated *P. entellus* with *B. malayi* showed high eosinophilia, which rose with the increasing microfilaraemia. Choong and Mak[26] observed the same phenomenon while working with the *B. malayi*–*P. cristata* (silvered leaf monkey) model.

Regarding correlation between parasitological findings and alteration in immunological parameters after therapy, it was observed that though DEC treatment resulted in elimination of parasites (both mf and adult worms), improvement in filaria-specific cellular hyporesponsiveness

could not be achieved immediately after treatment with higher doses of DEC. However, lower dose of DEC (18 mg/kg) showed some improvement in specific cellular response immediately after treatment. Investigators have reported that DEC could partially restore the filaria-specific proliferative response in humans with *W. bancrofti* infection[6,27]. T-cells from untreated infected, compound 82-437-treated infected and 200 mg/kg DEC-treated infected animals were unresponsive to ConA, while those from 18 mg/kg DEC-treated infected animals were responsive. It is known that T-cells of mf-positive monkeys show unresponsiveness to both filarial antigen and ConA[4]. Similar unresponsiveness to ConA has also been reported in other animal models[28,29] and in human subjects[30–32]. Several factors, including circulating immune complexes[33–35] have been implicated in this unresponsiveness. In addition, a marked depression in T-cell proliferative responses to mitogens has also been reported in human visceral leishmaniasis in monkeys[36]. In the present study DEC at 18 mg/kg dose level (which is equivalent to the human therapeutic dose of 6 mg/kg) indeed improved, as reported in human subjects, the T-cell response, thus clearly establishing the usefulness of the *P. entellus – B. malayi* system as a tertiary screening model. However, at 200 mg/kg, which is more than ten times the therapeutic dose equivalent, DEC suppressed the T-cell responsiveness, the mechanism and significance of which remains to be investigated. The decrease in CICs and filaria-specific IgG after therapy reflected the absence of parasites. However, after treatment with compound 82-437 there was less alteration in the immunological parameters both as regards to cell proliferation potential, levels of CICs and filaria-specific IgG.

Interestingly, there were no demonstrable histopathological alterations in the lymphoid organs of DEC-treated animals, reflecting evidence of elimination of parasites. Nevertheless, adulticidal action of DEC is known in cats[37] and humans harbouring *B. malayi* infection. However, against *W. bancrofti* the adulticidal action of DEC is doubtful. It acted mildly in repairing structural damage to the lymphatic system even in those individuals with preclinical disease[38]. This was explained to be due to repeated exposure of infection in humans. The adulticidal efficacy of the compound 82-437 was comparable to that reported in the rodent model[5]. Histological evidence of a few intact worms and granulomas around degenerating worms in the lymphoid tissues of the animals further confirmed the predictability of antifilarial efficacy in this model. Mak and his group[39] have extensively used the leaf monkey (*Presbytis* spp.) with sub-periodic *B. malayi* or *W. kalimantani* for tertiary evaluation and pharmacokinetic studies of compounds that have shown effectiveness in the primary and secondary screens. However, their model does not develop any limb oedema or episodic febrile attacks.

The present findings demonstrated that the Indian leaf monkey – *B. malayi* model responds to treatment with known

antifilarials in a predictable manner and may therefore be used as a tertiary screen for potential antifilarials. Further, direct correlation between alterations in parasitological profile and the AEC, as well as immunological and histopathological parameters following treatment, strengthened the predictability of the model for screening of potential antifilarials. The model may also be useful for testing potential compounds against filaria-mediated disease manifestations, particularly limb oedema.

1. Murthy, P. K., Tyagi, K. and Chatterjee, R. K., Indian langur (*Presbytis entellus*) as experimental host for *Brugia malayi* infection. *Curr. Sci.*, 1990, **59**, 1236–1239.
2. Tyagi, K., Murthy, P. K. and Chatterjee, R. K., *Brugia malayi* in Indian leaf monkey (*Presbytis entellus*): Response to repeated exposures of infective larvae. *Curr. Sci.*, 1996, **70**, 164–167.
3. Murthy, P. K., Tyagi, K., Ghosh, R. P., Murthy, P. S. R. and Chatterjee, R. K., Longitudinal humoral immune responses of Indian leaf monkey (*Presbytis entellus*) to *Brugia malayi* infection. *Parasitology*, 1999, **119**, 53–60.
4. Ghosh, R. P., Murthy, P. K., Tyagi, K., Murthy, P. S. R. and Chatterjee, R. K., Longitudinal cellular immune responses in symptomatic and asymptomatic Indian leaf monkey, *Presbytis entellus*. *J. Parasitol.*, 1999, **85**, 861–866.
5. Abuzar, S. *et al.*, Studies on potential filaricides-18: synthesis of 2,2'-disubstituted-5-5'-dibenzamidazolylketones and related compounds as potential anthelmintics. *J. Med. Chem.*, 1986, **29**, 1296–1299.
6. Piessens, W. F. *et al.*, Effect of treatment with diethylcarbamazine on immune responses to filarial antigens in patients infected with *Brugia malayi*. *Acta Trop.*, 1981, **29**, 227–234.
7. Haarbrink, M., Terhell, A. J., Abadi, G. K., Mitsui, Y. and Yazdanbakhsh, M., Inflammatory cytokines following diethylcarbamazine (DEC) treatment of different clinical groups in lymphatic filariasis. *Trans. R. Soc. Trop. Med. Hyg.*, 1999, **93**, 665–672.
8. Steel, C., Lujan-Trangy, A., Gonzales-Peralta, C., Zea-Flores, G. and Nutman, T. B., Immunologic responses to repeated ivermectin treatment in patients with onchocerciasis. *J. Infect. Dis.*, 1991, **164**, 581–587.
9. Murthy, P. K., Tyagi, K., Roychowdhury, T. K. and Sen, A. B., Susceptibility of *Mastomys natalensis* ('GRA' strain) to a subperiodic strain of human *Brugia malayi*. *Indian J. Med. Res.*, 1983, **77**, 623–630.
10. Dacie, Sir, J. V. and Lewis, S. M., Practical haematology. In *Basic Haematological Techniques*, Churchill Livingstone, London, 1995, 8th edn, pp. 59–63.
11. Buckley, J. J. C. and Edeson, J. F. B., On the morphology of *Wuchereria* sp. (malayi) from a monkey (*Macaca irus*) and from cats in Malayi, and on *Wuchereria pahangi* n. sp. from a dog and a cat. *J. Helminthol.*, 1956, **30**, 1–20.
12. Tandon, A., Murthy, P. K., Saxena, R. P., Sen, A. B. and Saxena, K. C., Dot-ELISA for diagnosis of lymphatic filariasis. *Indian J. Med. Res.*, 1988, **87**, 429–433.
13. Murthy, P. K., Murthy, P. S. R., Tyagi, K. and Chatterjee, R. K., Fate of implanted infective larvae of *Brugia malayi* in the peritoneal cavity of *Mastomys natalensis* and *Meriones unguiculatus*. *Folia Parasitol.*, **44**, 302–304.
14. Lowry, O. H., Rosebrough, N. R., Farr, A. L. and Randall, R. J., Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, 1951, **193**, 265–275.
15. Ottesen, E. A., Hiatt, R. A., Cheever, A. W., Sotomyor, Z. R. and Neva, F. A., The acquisition and loss of antigen specific cellular immune responsiveness in acute and chronic schistosomiasis in man. *Clin. Exp. Immunol.*, 1978, **33**, 38–47.

16. Bianco, C., Patrick, R. and Nussensued, G. V., A population of lymphocytes bearing a membrane receptor for antigen antibody complement complexes. *J. Exp. Med.*, 1970, **132**, 702.

17. Bloom, B. E., *In vitro Methods in Cell Mediated Immunity*. Academic Press, New York, 1977.

18. Voller, A., Barlett, A. and Bidwell, D. E., Enzyme immunoassays for parasitic diseases. *Trans. R. Soc. Trop. Med. Hyg.*, 1976, **70**, 98.

19. Murthy, P. K., Agarwal, A., Katiyar, J. C. and Sahib, M. K., Immune response patterns in different stages of active *Brugia malayi* infection in *Mastomys coucha*. *J. Parasit. Dis.*, 1995, **19**, 135–140.

20. Matsumura, K., Kazura, Y., Endo, R., Tanaka, K. and Inove, K., Detection of circulating immune complexes in the sera of dogs infected with *Dirofilaria immitis*, by Clq-binding enzyme linked immunosorbent assay. *J. Helminthol.*, 1986, **60**, 239–243.

21. Chatterjee, R. K., Fatma, N., Murthy, P. K., Sinha, P., Kulshreshtha, D. K. and Dhawan, B. N., Macrofilaricidal activity of the stem bark of *Streblus asper* and its major active constituents. *Drug Dev.*, 1992, **26**, 67–78.

22. Murthy, P. K., Nigar, Fatma and Chatterjee, R. K., Comparative susceptibility of ivermectin against different filarial parasites. CSIR Golden Jubilee Symposium, CDRI, Lucknow, 17–20 February 1992.

23. Borehem, P. F. L. and Atwell, R. B., Absence of shock-like reactions to ivermectin in dogs infected with *Dirofilaria immitis*. *J. Helminthol.*, 1985, **57**, 279–281.

24. Sim, B. K. L., Mak, J. W. and Kwa, B. H., Ultrastructural study of antibody-dependent cell-mediated damage to *Brugia malayi* infective larvae *in vitro*. In (eds Mak, J. W. and Yong, H. S.), *Immunology of Tropical Prarasitic Infection in Asian and Pacific Region*. Proceedings of the 25th Seameo-Tropmed Seminar, Kuala Lumpur, 19–21 October 1982, pp.11–19.

25. Gleich, G. J., Ottesen, E. A., Leiferman, K. M. and Ackerman, S. J., Eosinophils and human diseases. *Int. Arch. Allergy Appl. Immunol.*, 1989, **88**, 59–62.

26. Choong, M. F. and Mak, J. W., Hematological changes in subperiodic *Brugia malayi* infection of the leaf monkey, *Presbytis cristata*. *S.E. Asian J. Trop. Med. Pub. Health*, 1991, **22**, 168–170.

27. Lammie, P. J., Eberhard, M. L., Lieva, L. E., Lowrie Jr. and Katz, S. P., The effect of diethylcarbamazine treatment of bancroftian filariasis on the immunological reactivity of microfilaraemic individuals. *Trans. R. Soc. Trop. Med. Hyg.*, 1988, **82**, 726–729.

28. Klei, T. R., Mcvay, C. S., Dennis, V. A., Coleman, S. U., Enright, F. M. and Casy, H. W., *Brugia pahangi*: Effects of infection duration and parasite burden on lymphatic lesion severity, granulomatous hypersensitivity, and immune responses in jirds (*Meriones unguiculatus*). *Exp. Parasitol.*, 1990, **71**, 393–405.

29. Bosshardt, S. C., Coleman, S. U., Mcvay, C. S., Jones, K. L. and Klei, T. R., Impact of microfilaraemia on maintenance of a hyporesponsive cellular immune response in *Brugia*-infected gerbils (*Meriones unguiculatus*). *Infect. Immun.*, 1995, **63**, 940–945.

30. Ottesen, E. A., Weller, P. F. and Heck, L., Specific cellular immune unresponsiveness in human filariasis. *Immunology*, 1977, **33**, 413–421.

31. Piessens, W. F., Ratiwayanto, S., Tuli, S., Palmeri, J. H., Piessens, P. W., Koiman, I. and Dennis. D. T., Antigen specific suppressor cells and suppressor factors in human filariasis with *Brugia malayi*. *N. Engl. J. Med.*, 1980, **302**, 833–837.

32. Piessens, W. F. *et al.*, Antigenic specific suppressor T lymphocytes in human lymphatic filariasis. *N. Engl. J. Med.*, 1982, **307**, 144–148.

33. Todd, C. W., Goodgame, R. W. and Colley, D. G., Immune responses during human *schistosomiasis mansoni*. V. Suppression of schistosome antigen-specific lymphocyte blastogenesis by adherent/phagocytic cells. *J. Immunol.*, 1979, **122**, 1440–1447.

34. Prasad, G. B. K. S. and Harinath, B. C., Detection of filarial antigen in immune complexes in bancroftian filariasis by ELISA. *Indian J. Med. Res.*, 1983, **78**, 780–783.

35. Prasad, G. B. K. S., Kharat, I. and Harinath, B. C., Suppression of ConA induced lymphocyte transformation by sera from filarial patients with clinical manifestations. *Indian J. Exp. Biol.*, 1991, **29**, 352–354.

36. Dube, A., Srivastava, J. K., Sharma, P., Chaturvedi, A., Katiyar, J. C. and Naik, S., *Leishmania donovani*: cellular and humoral immune responses in Indian langur monkeys, *Presbytis entellus*. *Acta Trop.*, 1999, **73**, 37–48.

37. Edeson, J. F. B. and Laing, A. B. C., Studies on filariasis in Malaya: the effect of diethylcarbamazine on *Brugia malayi* and *B. pahangi* in domestic cats. *Ann. Trop. Med. Parasitol,*, 1959, **53**, 394–399.

38. Freedman, D. O., Buithuy, J. P. O., Filaio, D. E A., Braga, S., Silva, M. C. M. E., Maciel, A. and Furtado, A. E., Lymphoscintigraphic assessment of the effect of diethylcarbamazine treatment on lymphatic damage in human bancroftian filariasis. *Am. J. Trop. Med. Hyg.*, 1995, **52**, 258–261.

39. Mak, J. W., Navaratnam, V. and Ramachandran, C. P., Experimental chemotherapy of lymphatic filariasis. A review. *Ann. Trop. Med. Parasitol.*, 1991, **85**, 131–137.