population and deploying genetic variability through extensive gene flow, although neither the time frame nor the direction can be determined currently from the available data.

On the basis of the present study, it is concluded that the Indian population of rice blast fungus is genetically heterogeneous and the interrelationships amongst the different isolates can be easily, precisely and reliably explained by RAPD.

1. Ou, S. H., *Rice Diseases*, Commonwealth Mycological Institute, Kew, UK, 1985, pp. 109–201.
2. Maclean, J., *Rice Almanac*, International Rice Research Institute, Los Banos, Philippines, 1997.
3. McDonald, B. A., McDermott, J. M. and Goodwin, S. B., The population biology of host pathogen interactions. *Annu. Rev. Phytopathol.*, 1989, **27**, 77–94.
4. Levy, M., Romao, J., Marcheitti, M. A. and Hamer, J. E., DNA fingerprinting with dispersed sequence resolve pathotype diversity in the rice blast fungus. *Plant Cell*, 1991, **3**, 95–102.
5. Levy, M., Correa-Victoria, F. J., Zeigler, R. S., Xu, S. and Hamer, J. E., Genetic diversity of the rice blast fungus in the screening nursery in Columbia. *Phytopathology*, 1993, **83**, 1427–1433.
6. Chen, D., Zeigler, R. S., Leung, H. and Nelson, R. J., Population structure of *Pyricularia oryzae* at two screening sites in the Philippines. *Phytopathology*, 1995, **85**, 1011–1020.
7. Shull, V. and Hamer, J. E., Genetic differentiation in the rice blast fungus revealed by the distribution of *Magnaporthe grisea*: Genetic map, electrophoretic karyotype and occurrence of repeated DNAs. *Fungal Genet. Biol.*, 1996, **20**, 59–69.
8. Kumar, J., Nelson, R. J. and Zeigler, R. S., Population structure and dynamics of *M. grisea* in the Indian Himalayas. *Genetics*, 1999, **152**, 971–984.
9. Roumen, E., Levy, M. and Nottegham, J. L., Characterization of the European pathogen population of *Magnaporthe grisea* by DNA fingerprinting and pathotype analysis. *Eur. J. Plant Pathol.*, 1997, **103**, 363–371.
10. Correll, J. C., Harp, T. L., Guerber, J. C., Zeigler, R. S., Liu, B., Cartwright, R. D. and Lee, F. N., Characterisation of *Pyricularia grisea* in the United States using independent and molecular markers. *Phytopathology*, 2000, **90**, 1396–1404.
11. Viji, G., Gnanamanickam, S. S. and Levy, M., DNA polymorphism of isolates of *Magnaporthe grisea* from India that are pathogenic to fingermillet and rice. *Mycol. Res.*, 2000, **104**, 161–167.
12. Vakalounakis, D. J. and Fragkiadakis, G. A., Genetic diversity of *Fusarium oxysporum* isolates from cucumber: Differentiation by vegetative compatibility, pathogenicity and RAPD fingerprinting. *Phytopathology*, 1999, **89**, 161–168.
13. Kolmer, J. A. and Liu, J. Q., Virulence and molecular polymorphisms in international collections of the wheat leaf rust fungus *Puccinia triticina*. *Phytopathology*, 2000, **90**, 427–436.
14. Welsh, J. and McClelland, M., Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Res.*, 1990, **18**, 7213–7218.
15. Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A. and Tingey, S. V., DNA amplified by arbitrary primers are useful genetic markers. *Nucleic Acids Res.*, 1990, **18**, 6531–6535.
16. Annamalai, P., Ishii, H., Lalithakumari, D. and Revathi, R., Polymerase chain reaction and its applications in fungal disease diagnosis. *J. Plant Dis. Prot.*, 1995, **102**, 91–104.
17. Lima, A., Population genetic diversity of *P. grisea* on rice in Portugal. In Minutes of the 2nd Biennial Meeting of the Portuguese Phytopathology Society, Portugal, 1999, pp. 47–55.
18. Brunk, C. F., Jones, K. C. and James, T. W., Assay for nanogram quantities of DNA in cellular homogenates. *Anal. Biochem.*, 1997, **92**, 497–500.
19. Xia, J. Q. and Correll, J. C., DNA fingerprinting to examine micro-geographic variation in the *Magnaporthe grisea* (*Pyricularia grisea*) population in two rice fields in Arkansas. *Exp. Mycol.*, 1995, **19**, 171–177.
20. Han, S. S., Ra, D. S. and Nelson, R. J., Comparison of phylogenetic trees and pathotypes of *Pyricularia oryzae* in Korea. *J. Agric. Sci.*, 1993, **35**, 315–323.
21. Yamasaki, Y. and Niizeki, H., Studies on variation of the rice blast fungus *Pyricularia oryzae* Cav. I. Karyological and genetical studies on variation. *Bull. Natl. Inst. Agric. Sci.*, 1965, **13**, 231–274.
22. Zeigler, R. S., Recombination in *Magnaporthe grisea*. *Annu. Rev. Phytopathol.*, 1998, **36**, 249–276.
23. Valent, B., Crawford, M. S., Weaver, C. G. and Chumley, F. G., Genetic studies of fertility and pathogenicity in *Magnaporthe grisea*. *Iowa State J. Res.*, 1986, **60**, 569–594.
24. Ikeda, K., Nakayasiiki, H., Takagi, M., Tosa, Y. and Mayama, S., Heat shock, copper sulphate and oxidative stress activate the retrotransposon *MAGGY* resident in the plant pathogenic fungus *M. grisea*. *Mol. Genet. Genomics*, 2001, **266**, 318–325.
25. Kim, Nam-S., Park, Nam-Il., Kim, S.-H., Han, Sung, S. and Kang, K., Isolation of TC/AG repeat microsatellite sequences for the fingerprinting rice blast fungus and their possible horizontal transfer to plant species. *Mol. Cell*, 2001, **10**, 127–134.

ACKNOWLEDGEMENT. We thank Dr K. K. Muralidharan, Directorate of Rice Research, Hyderabad, for providing isolates of *M. grisea*.

Received 30 October 2003; revised accepted 7 January 2005

Brugia malayi infection: Correlation between chronicity of infection and lymphoid cell proliferation and DNA damage of host

M. A. Khan[1], S. Dixit[1], R. L. Gaur[1], J. K. Saxena[2], P. S. R. Murthy[3] and P. K. Murthy[1,*]

[1]Division of Parasitology, [2]Division of Biochemistry and [3]Division of Toxicology, Central Drug Research Institute, Lucknow 226 001, India

The present study was undertaken to investigate whether the hyporesponsiveness of the host induced by filarial infection was due to suppression of cell proliferation or DNA damage or both, of splenocytes or lymph node cells and its relation to chronicity of infection. Lymph node cells and splenocytes from *Mastomys coucha* infected with *Brugia malayi* at 1–2 months (amicrofilaraemic), 6–7 months (microfilaraemic) and >12 months (chronic microfilaraemic) infection were studied by Comet assay and lymphocyte transformation test. Cells from all chronically infected animals showed both DNA damage and suppressed proliferative response to filarial antigen/ConA, while this was seen only in 75% of

*For correspondence. (e-mail: psr_murthy@yahoo.com)

animals with six-month-old infection. About 18% of animals with 1–2-month-old infection showed DNA damage only. Splenocytes from early and mid-stage infected animals showed less proliferative response to parasite antigen or ConA compared to uninfected animals. Thus the findings show a significant correlation between DNA damage/suppressed cell-proliferative response and the chronicity of infection, and this may reflect on the development of hyporesponsiveness. In conclusion, the present findings suggest that in chronic infection in *M. coucha*, the parasite products may cause suppression of cell-proliferative responses and cytotoxicity/DNA damage in lymphocytes/splenocytes. This may collectively contribute to the development of hyporesponsiveness of the host.

LYMPHATIC filarial worms are complex multicellular organisms with prolonged survival in the human host, leading to down-regulation of antigen-specific proliferative response. It is well documented that patent infection with *Wuchereria bancrofti* or *Brugia malayi* is associated with reduction in both humoral and cellular responsiveness to filarial antigens[1–5], but treatment with diethylcarbamazine (DEC) partially restored the antigen-specific responses. However, some amicrofilaraemics or chronic symptomatic cases from endemic areas also exhibited similar down-regulation of antigen-specific proliferative response and restoration in responsiveness after DEC treatment[6]. Lammler *et al.*[7] and Murthy *et al.*[8] have demonstrated decrease in the number of lymphocytes and polymorphs (leucopenia) in *Mastomys coucha* (=*natalensis*) – *Litomosoides carinii* and Indian cat – *B. malayi* models respectively. Several investigators have demonstrated unresponsiveness to both mitogen and antigen in humans[1,3,9] and monkey models of filariasis[10] and leishmaniasis[11]. Many factors, including circulating immune complexes[12–14] have been implicated in this unresponsiveness. Recently, Nutman and Kumaraswami[15] have reported that microfilaraemic individuals showing diminished cellular proliferative responsiveness had decreased the number of positive CD4+ cells following *in vitro* exposure to filarial antigen. They suggested that apoptotic pathways might play a part in *in vivo* tolerance. However, whether filarial infection induces DNA damage in host cells is not known. The present study was therefore undertaken to investigate whether the hyporesponsiveness of the host induced by filarial infection was due to suppression of cell proliferation or DNA damage or both, in splenocytes or lymph node cells and its relation to chronicity of infection.

M. coucha harbouring *B. malayi* infection was used[16] as the model.

Groups I, II and III consisted of 11, 4 and 6 animals having early, i.e. 1–2 months post-larval inoculation (p.i.), mid (6–7 months p.i.) and chronic (more than 12 months p.i.) stages of *B. malayi* infection respectively. Animals of Groups II and III showed circulating microfilariae (mf). Groups IV and V comprised 3 and 8 animals respectively. Cells of Group IV were treated with 200 μM H_2O_2 for 10 min and kept as positive control. Cells of Group V were left as such and considered as negative control.

Groups I–III and V consisted of pooled data of repeat experiments using 2–4 animals in each experiment. Group IV also consisted of pooled data of repeat experiments using one animal in each experiment.

Adult worms and mf were recovered from the peritoneal cavity of experimentally infected jirds (*Meriones unguiculatus*). The parasites were washed several times with sterile PBS before use. Soluble parasite extracts were prepared using the method of Tandon *et al.*[17]. Briefly, the worms were crushed in PBS (pH 7.2) followed by sonication at 10 Kc for 10 min with 60 s interval after every stroke of 60 s. The homogenate thus obtained was centrifuged at 5000 rpm for 30 min at 4°C. The supernatant was collected and protein content estimated using the method of Lowry *et al.*[18].

Lymph nodes and spleens were removed from the animals under aseptic condition and kept in sterile RPMI 1640 medium. These organs were washed 3–4 times with sterile medium containing penicillin 100 U/ml and streptomycin 100 μg/ml. Cells were isolated according to the method described by Klei *et al.*[19]. Briefly, cells were freed by gentle teasing of lymph nodes or spleens and strained through 100 μm porosity of nylon cell strainer to obtain clean cells. RBCs contaminated in spleen cells were lysed with 0.87% NH_4Cl. The cell suspension thus obtained was centrifuged at 2000 rpm at 4°C for 5–7 min followed by three washes with medium and re-suspended in complete RPMI 1640 medium. The viability of the cells was checked using 0.1% trypan blue. Viable cells were counted and adjusted in such a way that the final concentration of cells in the culture well became 2×10^6/ml concentration.

Lymphocyte transformation test was carried out using ^3H thymidine uptake[10]. Lymphocytes or splenocytes (2×10^6/ml) were cultured in RPMI 1640 medium supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml), 2 mM L-glutamine and 10% foetal bovine serum in 96-well flat bottom culture plate (Nunc, Denmark) in triplicate with or without parasite extract (20 μg/ml) or ConA (10 μg/ml). Cultures were incubated at 37°C in humid atmosphere (5% CO_2 and 95% air) for 72 h. The cells were then pulse labelled with ^3H thymidine (1 μci/well) and harvested after 18 h in a cell harvester. ^3H thymidine incorporation was counted in liquid scintillation β-counter. Results were expressed in terms of stimulation index, which was calculated by dividing mean cpm of stimulated culture with mean of unstimulated culture.

MTT assay was used to determine the cytotoxic potential of *B. malayi* adult parasite extract as described by Kayser *et al.*[20]. Briefly, splenocytes (2×10^6/ml) from naïve *M. coucha* were incubated with or without parasite extract at 37°C in humid atmosphere (5% CO_2 and 95% air) for 72 h. After incubation, the cells were centrifuged at 3000 rpm for 15 min and 0.5% MTT prepared in PBS was then added to the pellet and incubated at 37°C for 2 h followed

by centrifugation at the same speed. The pellet containing formazan was extracted in DMSO for 1 h. OD of soluble formaza was read at 510 nm.

For assessment of DNA damage, Comet assay was performed according to Singh *et al.*[21], with some modifications. Briefly, cell pellets were mixed with 0.5% low-melting temperature agarose at 37°C. The cell suspension was then loaded in wells of 0.8% high melting agarose cast on horizontal plate. Each well charged with 1×10^6 cells was covered with a thin layer of 0.8% normal melting agarose, cooled and immersed in a lysing solution containing 1% Sarcosinate (Sigma, St. Louis, MO), 2.5 M NaCl, 100 mM sodium EDTA, 10 mM Tris (pH 10.0) and 1% Triton X-100 for 2–3 h at 37°C. The set was removed and placed in a horizontal gel electrophoresis unit filled with freshly prepared electrophoretic buffer (1 mM sodium EDTA, 200 mM NaOH; pH 14.0) and allowed to stand for 20 min. Electrophoresis was run for 2 h at 25 V and 300 mA. After electrophoresis, the gel was washed with 0.4 M Tris (pH 7.5) to remove excess alkali. Slides were then stained with ethidium bromide and observed under UV Spectromax gel documentation. The damaged cells appeared as smear extended towards the anode. Length of migration reflected the extent of DNA breakage in the cell[21].

Results were presented as mean ± SD of two to three experiments and the data were analysed statistically using GraphPad Prism®. Differences with $P < 0.05$ were considered significant.

The level of DNA damage in the splenocytes and lymph node cells of *M. coucha* (uninfected and *B. malayi* infected) was assessed on the basis of migration of DNA (Table 1). Lymph node cells from normal animals (Group IV) treated *in vitro* with H_2O_2 showed DNA damage as regards to DNA migration (1.5 cm). In contrast, unexposed cells of lymph nodes or spleen (Group V) did not show any DNA damage. All the six chronically infected animals of Group III (more than 12-month-old infection) showed DNA damage (in the form of smear) in both splenocytes (1.17 cm ± 0.75) and lymph node cells (0.65 cm ± 0.28). However, three of the four animals (Group II) harbouring 6–7-month-old infection with mf in the peripheral blood showed substantially low damage in DNA of splenocytes (0.3 cm ± 0.09) only. Interestingly, nine out of 11 animals (Group I) having early stage of infection (1–2-month-old infection) with no peripheral mf showed no damage in DNA of splenocytes or lymph node cells. However, the extent of damage in DNA of lymph node cells of the remaining two animals of this group was comparable to the damage observed in Group II animals. Apparently, there was a direct correlation between extent of DNA migration and duration of exposure (Table 1).

Both splenocytes and lymph node cells of chronically infected animals showed significantly suppressed ($P < 0.01$) proliferative responses to both filarial antigen and ConA. Splenocytes of animals harbouring early and mid-stage of infection showed less responsiveness to parasite antigen compared to uninfected animals. However, splenocytes of animals of mid-stage infection showed suppressed response to ConA also (Table 2). Interestingly, *B. malayi* adult parasite extract exerted significant ($P < 0.0001$) cytotoxic effect as assessed by MTT assay on splenocytes of naïve *M. coucha* when incubated *in vitro* (Table 3).

Chronic exposure with any infectious agents or toxic substances is known to lead to DNA damage in host cells[22–24]. Our results show preliminary but definite findings that *B. malayi* infection may induce DNA damage in splenocytes or lymph node cells of mf-positive animals and that chronic stage of filarial infection induced significant damage in DNA. It also appeared that extent of DNA damage was significantly dependent on the length of exposure of the host to parasites, as all the chronically infected animals showed DNA damage and only a small percentage (18%) of animals with early stage of infection exhibited such damage. The smear-type damage in DNA of filaria-infected cells was comparable to H_2O_2-mediated DNA damage in lymph node cells or splenocytes of normal animals. Our present

Table 1. DNA damage assessed by electrophoretic mobility in splenocytes and lymph node cells of *Mastomys coucha* harbouring different stages of *Brugia malayi* infection (values are mean ± SD)

Group (*n*)	Duration of infection	Type of lymphoid cell	No. of animals showing DNA damage in cells	Length of DNA migration (cm)
I (11)	Early infection (1–2 months; mf negative)	Lymph node cells	2	0.4 and 0.5
		Splenocytes	0	No smear or ladder
II (4)	Mid infection (6–7 months; mf positive)	Lymph node cells	0	No smear or ladder
		Splenocytes	3	0.3 ± 0.09**
III (6)	Chronic infection (12 months)	Lymph node cells	6	0.65 ± 0.28**
		Splenocytes	6	1.17 ± 0.75#
IV (3)	Control uninfected (H_2O_2 treated)	Lymph node cells	3	1.5 ± 0.30#
V (8)	Control uninfected	Lymph node cells	0	No smear or ladder
		Splenocytes	0	No smear or ladder

n, No. of animals used.
**$P < 0.01$ (over untreated control group).
#$P < 0.01$ (over Groups II and III).

Table 2. Proliferative response of splenocytes and lymph node cells of *M. coucha* harbouring different stages of *B. malayi* infection to filarial parasite antigen and ConA (values are mean ± SD)

Group (*n*)	Duration of infection	Type of lymphoid cell	Stimulation index	
			B. malayi adult worm extract	ConA
I (4)	Early infection (1–2 months; mf negative)	Lymph node cells	5.25 ± 0.08	5.87 ± 0.88
		Splenocytes	2.26 ± 0.37**	4.18 ± 0.41
II (4)	Mid-infection (6–7 months; mf positive)	Lymph node cells	5.47 ± 2.90	5.45 ± 1.40
		Splenocytes	3.16 ± 0.7*	2.26 ± 0.64**
III (6)	Chronic infection (>12 months)	Lymph node cells	2.25 ± 0.07**	1.4 ± 0.17**
		Splenocytes	1.75 ± 0.26**	2.3 ± 0.21**
V (8)	Control uninfected	Lymph node cells	5.56 ± 0.04	4.25 ± 0.08
		Splenocytes	4.49 ± 0.05	5.89 ± 0.38

n, No. of animals.
*$P < 0.05$ (over untreated control group).
**$P < 0.01$ (over untreated control group).

Table 3. Cytotoxicity of *B. malayi* adult worm extract to splenocytes from naïve *M. coucha* assessed by MTT assay (values are mean ± SD)

	OD_{510}	*P* value
B. malayi adult worm extract	0.81 ± 0.25	0.0001
Control	1.85 ± 0.07	

findings show that DNA damage in the splenocytes or lymph node cells of the animals was the consequence of chronic parasite exposure, particularly to mf stage and that the characteristic smear-type DNA tailing seen in our study suggests cell necrosis. In bancroftian filarial patients, several investigators consistently found necrotic cells in lymph-node biopsies. Histopathological observations in both human and animals with chronic filarial infection revealed focal necrotic regions in the lymph nodes and spleen[25]. Thus the data support the view that both suppressed cell proliferation and necrotic depletion of lymphocytes by the parasite molecules/antigens might precipitate the development of unresponsiveness or hyporesponsiveness in filarial infected hosts. Recently, Jenson *et al.*[26] demonstrated that *Brugia* mf could selectively induce lymphocyte apoptosis and suggested that apoptosis can contribute to the defect in proliferative response of the cells to parasite extract and provide favourable environment for persistence of mf. However, they mentioned that these findings warrant further investigations. In the present study it was observed that mf was not solely responsible for DNA damage, as filarial-specific cellular hyporesponsiveness was detected in animals harbouring early stage of infection, which had no mf either in circulation or in the uteri of female worms. This suggests that other stages of the parasite or their molecules or some unidentified factors may also play a role in damaging DNA in host cells. Holzmuller *et al.*[24] demonstrated NO-mediated cytostatic and cytotoxic effects on axenically grown and intracellular *Leishmania amazonensis* amastigotes consequent to activation of a pathway that was referred to as apoptosis-like process. Melino *et al.*[27] have reported that NO could induce both necrosis as well as apoptosis. We have observed that peritoneal macrophages from *M. coucha* harbouring early stage of *B. malayi* infection when re-stimulated with *B. malayi* antigen *in vitro*, significantly more NO was found in culture supernatants compared to cells from mid- and chronic stages of infection (unpublished observation). It is therefore expected that constant release of NO at the early stage of infection may predispose to more DNA damage during chronic stage of infection. Perhaps, in the present study, due to this reason reduced level of cellular proliferative response was observed during early stage of infection too. Jenson *et al.*[26] reported that mf-infected mice, which produced Th1 response were unable to proliferate in the presence of high level of NO, but they have also reported that CD4+ T cells were the major source of Th2 cytokines following L_3 infection. In our previous study with Indian leaf monkey (*Presbytis entellus*) infected with *B. malayi*, we have shown a positive correlation between presence of CD4+ T cells and suppressed cell-mediated immune response in animals that developed disease manifestations[10]. Nevertheless, NO is not only identified as an immunoprotective molecule of innate immunity defense system against invading pathogens, it also acts as molecular cytotoxin, causes iron loss, inhibits DNA synthesis and mitochondrial respiration which may lead to necrosis, programmed cell death and may ultimately lead to immuno-suppression. In addition, we have also observed that adult parasite extract induces apoptosis in lymph node cells *in vitro* (unpublished observation). Therefore, both apoptosis and necrosis may contribute to the cellular unresponsiveness or hyporesponsiveness in the host. In the jird–*B. pahangi* model, Prier and Lammie[28] and Leiva and Lammie[29] correlated hyporesponsiveness to suppression of cell proliferation by parasite antigen. In the present study, hyporesponsiveness appeared to be due to both suppression of cell proliferation of splenocytes and lymph node cells and cytotoxicity to splenocytes in the presence

of parasite extract. It thus fortifies the concept of hyporesponsiveness and provides evidences for mechanism(s) through which the parasite may bring about this hyporesponsiveness in the host. The present findings show a significant correlation between DNA damage/cell proliferative response and the chronicity of infection, and this may reflect in turn on the development of hyporesponsiveness in the host.

In conclusion, the present findings suggest that in chronic infection in *M. coucha*, the parasite products may cause suppression of cell-proliferative responses and cytotoxicity/DNA damage in lymphocytes/splenocytes, and this may collectively contribute to the development of hyporesponsiveness of the host.

1. Ottesen, E. A., Weller, P. F. and Heck, L., Specific cellular immune unresponsiveness in human filariasis. *Immunology*, 1977, **33**, 413–421.
2. Piessens, W. F., McGreevy, P. B., Piessens, P. W., Mcgreevy, M., Koiman, J., Sarsoso, H. S. and Dennis, D. T., Immune response in human infection with *Brugia malayi* specific cellular unresponsiveness to filarial antigen. *J. Clin. Invest.*, 1980, **65**, 172–179.
3. Piessens, W. F., Ratiwayanto, S., Tuti, S., Palmieri, J. H., Piessens, P. W., Koiman, J. 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.
4. Nutman, T. B., Kumaraswami, V., Rao, L., Narayanan, P. R. and Ottesen, E. A., An analysis of *in vitro* B-cell immune responsiveness in human lymphatic filariasis. *J. Immunol.*, 1987, **138**, 3954–3959.
5. Piessens, W. F., Wadee, A. and Kurniawan, L., Regulation of immune responses in lymphatic filariasis. In *Filariasis. Ciba Foundation Symposium 127* (eds Evered, D. and Clark, S.), John Wiley, UK, 1987, pp. 164–173.
6. Lammie, P. J., Leiva, L. E., Ruff, A. J., Eberhard, M. L., Lowrie, R. C. and Katz, S. P., Preliminary characterization of immunologic responsiveness of microfilaraemic individuals. *Am. J. Trop. Med. Hyg.*, 1988, **38**, 125–129.
7. Lammler, G., Gruner, D. and Zahner, H., Chemotherapeutic studies on *Litomosoides carinii* infection of *Mastomys natalensis*. 2. Alteration of haematological parameters after the administration of filaricidal compounds. *Z. Tropenmed. Parasitol.*, 1975, **26**, 98–110.
8. Murthy, P. K., Roychowdhury, T. K. and Sen, A. B., Sequential changes in leucocytes of cats during infection with sub-periodic *Brugia malayi*. *Indian J. Parasitol.*, 1980, **4**, 233–235.
9. Piessens, W. F. *et al.*, Antigen-specific suppressor T lymphocytes in human lymphatic filariasis. *N. Engl. J. Med.*, 1982, **307**, 144–148.
10. Ghosh, R. P., Murthy, P. K., Tyagi, K., Murthy, P. S. R. and Chatterjee, R. K., Longitudinal cellular immune responses in asymptomatic and symptomatic *Brugia malayi* infected Indian leaf monkey *Presbytis entellus*. *J. Parasitol.*, 1999, **85**, 861–866.
11. Dube, A., Srivastava, J. K., Sharma, P., Chaturvedi, A., Katiyar, J. C. and Naik, S., *Leihsmania donovani*: Cellular and humoral immune responses in Indian langur monkeys, *Presbytis entellus*. *Acta Trop.*, 1999, **73**, 37–48.
12. 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.
13. 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.
14. Prasad, G. B. K. S., Kharat, I. and Harinath, B. C., Suppression of ConA induced lymphocyte transformation by sera from filarial patients with clinical manifestation. *Indian J. Exp. Biol.*, 1991, **29**, 352–354.
15. Nutman, T. B. and Kumaraswami, V., Regulation of immune response in lymphatic filariasis: Perspectives on acute and chronic infection with *Wuchereria bancrofti* in South India. *Parasite Immunol.*, 2001, **23**, 389–399.
16. Murthy, P. K., Tyagi, K., Roychowdhury, T. K. and Sen, A. B., Susceptibility of *Mastomys natalensis* (GRA strain) to sub-periodic strain of *Brugia malayi*. *Indian J. Med. Res.*, 1983, **77**, 623–630.
17. Tandon, A., Murthy, P. K., Saxena, R. P., Sen, A. B. and Saxena, K. C., Dot–ELISA for diagnosis of human *Brugia malayi*. *Indian J. Med. Res.*, 1988, **87**, 429–433.
18. 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.
19. 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.
20. Kayser, O., Kiderlen, A. P., Folkens, U. and Kolodzief, H., *In vitro* leishmanicidal activity of Aurones. *Planta Med.*, 1999, **65**, 316–319.
21. Singh, N. P., McCoy, M. T., Tice, R. R. and Schneider, E. L., A simple technique for quantitation of low levels of DNA damage in individual cells. *Exp. Cell. Res.*, 1988, **175**, 184–191.
22. Mycroft, F. J. and Hiatt, P. H., The toxic hazards of industrial and occupational chemicals. In *Poisoning and Drug Overdose* (ed. Olsen, K. R.), Appleton & Lange, Norwalk, 1990, pp. 358–504.
23. Awara, W. M., El-Nabi, S. H. and El-Gohary, M., Assessment of vinyl chloride-induced DNA damage in lymphocytes of plastic industry workers using a single-cell gel electrophoresis technique. *Toxicology*, 1998, **128**, 9–16.
24. Holzmuller, P., Sereno, D., Cavaleyra, M., Mangot, I., Daulouede, S., Vincendeau, P. and Lemesre, Jean-Loup, Nitric oxide mediated proteosome-dependent oligonucleosomal DNA fragmentation in *Leishmania amazonensis* amastigotes. *Infect. Immun.*, 2002, **70**, 3727–3735.
25. Murthy, P. K., Tyagi, K., Murthy, P. S. R. and Chatterjee, R. K., 28th Annual Convention of Indian College of Allergy and Applied Immunology and CME, and Workshop on Recent Trends in Naso – Respiratory Allergic Disorders, Bikaner, 25–27 November 1994.
26. Jenson, S. J., O'Connor, Richard Osborne J. and Devaney, E., Infection with *Brugia* microfilariae induces apoptosis of CD+ T lymphocytes: A mechanism of immune unresponsiveness in filariasis. *Eur. J. Immunol.*, 2002, **32**, 858–867.
27. Melino, G., Bernassola, F., Knight, R. A., Corasaniti, M. T., Nistico, G. and Finazzi-Agro, A., S-nitrosylation regulates apoptosis. *Nature*, 1997, **388**, 432–433.
28. Prier, R. C. and Lammie, P. J., Differential regulation of *in vitro* humoral and cellular immune responsiveness in *Brugia pahangi*-infected jirds. *Infect. Immun.*, 1988, **56**, 3052–3057.
29. Leiva, L. E. and Lammie, P. J., Regulation of parasite antigen-induced T cell growth factor activity and proliferative responsiveness in *Brugia pahangi*-infected jirds. *J. Immunol.*, 1989, **142**, 1304–1309.

ACKNOWLEDGEMENTS. We thank the Director, CDRI, Lucknow for encouragement during the study. The award of a Senior Research Fellowship by CSIR, New Delhi to M.A.K. is acknowledged. Thanks are due to Mr V. K. Bose and Mr R. C. Rai for technical assistance.

Received 28 July 2004; revised accepted 6 January 2005