Inhibition of filarial proteases by antibodies from human filariasis

Lymphatic filariasis caused by *Wucheria bancrofti* is a major public health disease in India. Epidemiological studies indicate that people living in endemic regions acquired resistance to the disease after prolonged exposure to infection[1], although the direct evidence for immunity in human has been difficult to establish. Probably protective immune reactions to the parasite would be multifactorial – comprising both non-specific and specific (immunological) effector components. Elucidation of natural immune mechanisms operative in endemic population will help in devising better control strategies against the disease. Immunochemical characterization of antigens, especially those having biochemical functions (e.g. enzymes), can provide data relevant to the biology of parasites. Proteases of parasites are an important group of enzymes which are actively involved in various aspects of host–parasite interactions[2], for example in parasite nutrition, inactivation of host immune response, and invasion of host tissues. Antibodies inhibitory to proteases were detected in animals immune to or infected with the parasites[3,4], thereby emphasizing their importance. Antibody-mediated inhibition of protease activities may induce arrested growth of parasites and consequently benefit the host. Indeed, proteases have been implicated in conferring resistance to many diseases like malaria[5], trypanosomiasis[6], dictyocaulosis[4] and others[7,8].

Studies on immunological role of filarial proteases in endemic people have been initiated by us recently. They are described as allergen[9] and immunodiagnostic antigen[10] in *W. bancrofti*-infected individuals. Host inhibitory antibody response to filarial proteases, especially in humans, has remained unknown. Here we report the presence of such antibodies in filariasis and relate their generation to the severity of infection. The effect of antibodies isolated from different groups of filarial sera was studied on the protease activities in *W. bancrofti* infective larvae (L_3) that initiates human infection, adult extract (AE) of *Setaria digitata*, an immunoanalogue of human parasite and a protease fraction (Fr. III) purified from *Setaria*.

Filarial sera were collected from disease-free normal (endemic normals, EN) and infected individuals (symptomatic chronic patients, CP and asymptomatic microfilaraemics, AS) living in a filarial endemic village (Olosingh, Khurda district, Orissa). Pooled sera of different groups of filariasis were made by adding an equal volume of serum from individuals ($n = 20$ in each group). A control serum pool (NEN) was also prepared from residents of Koraput (non-filarial region) district of Orissa. IgG was purified from each serum pool by protein A-sepharose column.

Saline soluble homogenate of *W. bancrofti* L_3, *Setaria digitata* adult (AE) and an allergenic fraction with protease activity (Fr. III) were prepared as described before[10,11]. Protease activity was measured using azocoll[10], a general protease substrate. Samples (20 µl) were pre-incubated for 1 h at 37°C and 4 h at 4°C with IgG from filarial or non-endemic normal sera in 100 µl of assay buffer before performing protease assay with azocoll.

As shown in Table 1, IgG from chronic patients was the most effective in inhibiting protease activities in all preparations. IgG from AS sera partially inhibited activities in *W. bancrofti* L_3 and AE but not in Fr. III, indicating differences in antigenicity of the protease. IgG from EN and NEN (data not shown) sera was not inhibitory to the proteases.

These results indicate that neutralizing antibodies to filarial proteases were generated during natural course of human filariasis. The generation of inhibitory antibodies depends on the severity of infection. Thus chronic patients with elephantiasis and hydrocele have highest level whereas endemic normals, a group exposed to infection but non-infected, have undetectable level of these antibodies. Sera from asymptomatic microfilaraemic individuals, another infected group in filariasis, showed intermediate degree of inhibition. It would be worthwhile to find out what triggers the generation of such protease-neutralizing antibodies during filariasis – is it related to pathological changes or immune effector mechanisms.

Table 1. Antibody-mediated inhibition of filarial protease activity

Filarial group	Control activity remaining (%)		
	S. digitata (AE)	*W. bancrofti* L_3	Fr. III
Endemic normals (EN)	100	100	100
Asymptomatic microfilaraemic (AS)	60	65	100
Chronic filariasis (CP)	30	0	0

Protein A-sepharose purified antibody (60 µg) from filarial sera was incubated with parasitic extract (10–20 µg protein) prior to azocoll hydrolysis. The extent of inhibition was expressed as the percentage activity remaining relative to a control without IgG. The means of two independent assays are shown.

1. World Health Organization, TDR/FIL/SWG 1987, 13:87.3.
2. McKerrow, J. H., Sun, E., Rosenthal, P. J. and Bouvier, J., *Annu. Rev. Microbiol.*, 1993, **47**, 821–853.
3. Knox, D. P. and Kennedy, M. W., *Mol. Biochem. Parasitol.*, 1988, **28**, 207–216.
4. Britton, C., Knox, D. P., Canto, G. J., Urquhart, G. M. and Kennedy, M. W., *Parasitology*, 1992, **105**, 325–333.
5. Rosenthal, P. J., Lee, G. K. and Smith, R. F., *J. Clin. Invest.*, 1993, **91**, 1052–1056.
6. Authie, E., *Parasitol. Today*, 1994, **10**, 360–364.
7. Commins, A. A., Goodger, B. V. and Wright, G., *Int. J. Parasitol.*, 1985, **5**, 491–495.
8. Cox, G. N., Pratt, D., Hageman, R. and Boisvenue, R. J., *Mol. Biochem. Parasitol.*, 1990, **41**, 25–34.
9. Beuria, M. K., Bal, M. S. and Das, M. K., *J. Helminthol.*, 1995, **69**, 181–185.
10. Bal, M. S. and Das, M. K., *Curr. Sci.*, 1994, **67**, 1018–1019.
11. Das, M. K., Dash, A. P., Ravindran, B., Pattnaik, N. M. and Subramanyan, V. R., *Acta Tropica*, 1988, **45**, 387–388.

ACKNOWLEDGEMENTS. We thank the residents of Olosingh Village, Khurda, Orissa, for their cooperation.

M. S. BAL
M. K. DAS

Parasite Immunology Division,
Regional Medical Research Centre,
Chandrasekharapur,
Bhubaneswar 751 016, India