Functional Antigenic Molecules of Different Life-Stages of Rodent Filariid, *Acanthocheilonema viteae* and Their Cross Reactivity with Human Filarial (*Wuchereria bancrofti*) Sera.

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Abstract: Proteins obtained by SDS-solubilisation of various developmental stages (adults, uterus contents, microfilariae and infective larvae) of the filariid *Acanthocheilonema viteae* were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and electrophoretically transferred onto nitrocellulose filter sheets. The results showed minor differences in the protein pattern of adult worms, microfilariae, uterine contents and infective larvae. Immunoperoxidase staining of these antigens with infected mastomys sera showed that maximum number of antigens in infective larval stage were identified by the sera collected at prepatent stage of infection whereas in adult antigen maximum bands were recognized by sera from patent infection. A large number of microfilarial antigens were found to be immunoreactive during patent stage but only a few remained functional during latency. Uterine antigens possess a number of immunoreactive components which reacted with antibodies in sera of all stages of infection with maximum recognition by patent (microfilaraemic) serum. Cross reaction between *A. viteae* antigen and *W. bancrofti* human sera led to identification of a few common molecules which demonstrated the usefulness of antigens of rodent filariid *A. viteae* in immunodiagnosis or in evolving strategies for vaccination against human filariasis.

Key words: Acanthocheilonema viteae, Wuchereria bancrofti, Mastomys coucha, Ornithodoros moubata, antigen, cross-reaction, characterization, filaria, infective larvae, microfilariae, western blotting.

INTRODUCTION

Filarial parasites have a complex life-cycle involving various life-stages having both identical and dissimilar metabolic and antigenic make up. Investigations on the development of latency or acquired resistance of host to filarial infections were made earlier by Bagai and Subrahmanyam, 1970; Singh *et al* 1987, 1988; Lawrence *et al.* 1992) but the antigenic molecules responsible for the development of such response need in depth exploration. In vertebrate host, microfilariae (mf) and adult worms remain for prolonged period, however with a brief spell of other developing cycles which include L_3, L_4 and L_5 stages. All these life-stages exert antigenic stimuli eliciting immune responses to stage-specific as well as com-

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mon antigens. Characterization of antibody response of host to antigens of these life-forms would be useful in identifying molecules of diagnostic and prophylactic potentials. An attempt has been made in the present investigation to characterise antibodies directed against stage-specific molecules in mastomys with *Acanthocheilonema viteae* as well as to identify cross-reacting molecules of *A. viteae* and *Wuchereria bancrofti*.

Materials and Methods

Infection— Six week old *Mastomys coucha* (male) were infected with *A. viteae* by subcutaneous inoculation of 50 infective larvae (L_3) isolated from ticks *Ornithodoros moubata* as described earlier (Singh *et al.*, 1985).

Parasites— Adult worms of both sexes were isolated from the subcutaneous tissues of heavily infected mastomys 80 ± 10 days post L_3 exposure (p.e), washed in sterile medium RPMI 1640 (Sigma, USA) and kept frozen at -20C until use.

Microfilariae (mf) were separated from heparinized blood drawn through the retro-orbital plexus of anaesthetized microfilaraemic mastomys. Mf were isolated by membrane filtration (5.0μ) applying negative pressure (Singh *et al*, 1985) washed with the medium and kept at -20C.

Uterine contents of adult female parasites were collected by teasing the worms and isolating the uteri.

Infective larvae were isolated by dissecting infected ticks after 30 ± 1 day of infective blood feeding on anaesthetized donor. L_3 were collected free of tick tissue, washed several times with sterile medium and preserved frozen.

Sera— Blood for sera was taken from the retro-orbital plexus of 5 infected mastomys during prepatent period (30 and 50 days p.e.), patent (90 and 120 days p.e.) and latent (210 and 300 days p.e.) stages of infection. Sera were isolated and stored at -20C.

Blood samples were also taken from human subjects living in filaria-endemic area belonging to microfilaraemic, symptomatic and endemic normal groups.

SDS-PAGE— The test was performed according to Laemmli (1970) with modificatios of Ruppel and Cioli (1977) using 1.0mm thick linear gradient slab gel ($7-15\%$). Worms were homogenized and solubilized in sample buffer (1% w/v SDS; 5% w/v β-mercaptoethanol; 10% v/v glycerol in 63 mM Tris-HCl, pH 6.8). Protease inhibitors were earlier added to the sample buffer. Electrophoresis was carried out at 25 mA for $4-6$ hrs. in cold. Prestained markers (Sigma) and low and high molecular weight markers (Pharmacia) were simultaneously run as standard. Gel was stained with commassie blue, destained and photographed.

Western blotting— After completion of electrophoresis, separated proteins in gels were transferred onto nitrocellulose sheets (Towbin, 1974) overnight at 150 mA in cold and processed according to Burnette (1981). Nitrocellulose strips were cut, blocked with 3% BSA in Tris-buffered Saline (TBS, pH 7.2) and reacted with infected sera at room temperature for 1.5 hr. The second antibody (goat antimouse IgG^+ IgM-peroxidase conjugated, MEDAC, FRG) was added and reactions were visualized after adding 0.05% β-3'-diaminobenzidine (Sigma) containing 5×10^{-5}M H_2O_2 as substrate. The strips were air-dried and photographed.

RESULTS

SDS–PAGE profile

There were minor differences in the protein component on SDS-PAGE between adult worms, microfilariae and uterine contents while infective larvae had slightly different pattern. L_3 antigen was separated into 51 components while adult, mf and uterine contents had respectively 58, 65 and 53 protein bands (Fig. 1). The molecular weight ranged between 14 kDa and 200 kDa.

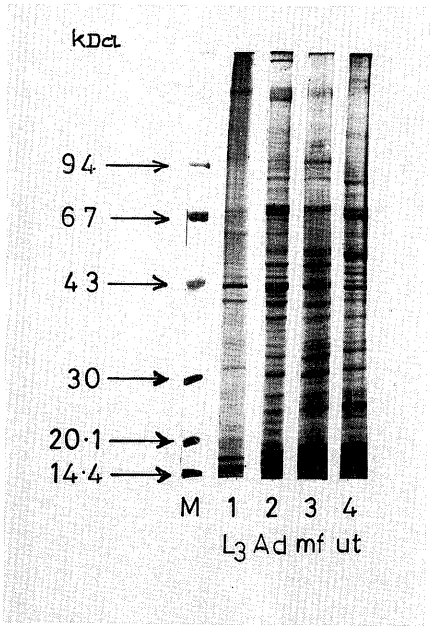

Fig. 1. SDS-PAGE profile of *A. viteae* antigens derived from, lane 1, infective larvae; lane 2, adult worms; lane 3, microfilariae; lane 4, uterine contents.

Identification of functional antigen

L_3 antigen

Maximum antigens were identified in blots in the L_3 stage when reacted against infected mastomys sera collected at prepatent stage of infection. Many of these bands started becoming faint or disappeared with the maturity of infection (Fig. 2a). Even 50 days old serum recognized much fewer L_3 protein molecules than sera of 30 days infection and very few could react with sera collected at very later stage of infection (370 days) and were mainly confined between 50 and 70 KDa. However, at the time of latency (210–300 days) many of the high molecular mass (Mr) components were immunoreactive (between 48.5 and 116 kDa). Nevertheless, few molecules elicited antibody response at all the stages of infection (84, 69 and 22 kDa). Lower Mr components like 40, 26, 21, 11, 9, 5 and 4 kDa reacted well with the prepatent sera and appear to be L_3 specific (Fig. 2a).

Adult antigens

The maximum and most intense reacting bands were recognized in adult parasites by sera from patent infection. A single band at 69 kDa and another thick one just below 36.5 kDa along with two other low Mr components were recognized exclusively by the patent but not prepatent or latent sera. Nevertheless, two immunodominant bands of 26.6 and 14 kDa identified by microfilaraemic sera (90−120 days) reacted only faintly with prepatent or latent sera (Fig. 2b).

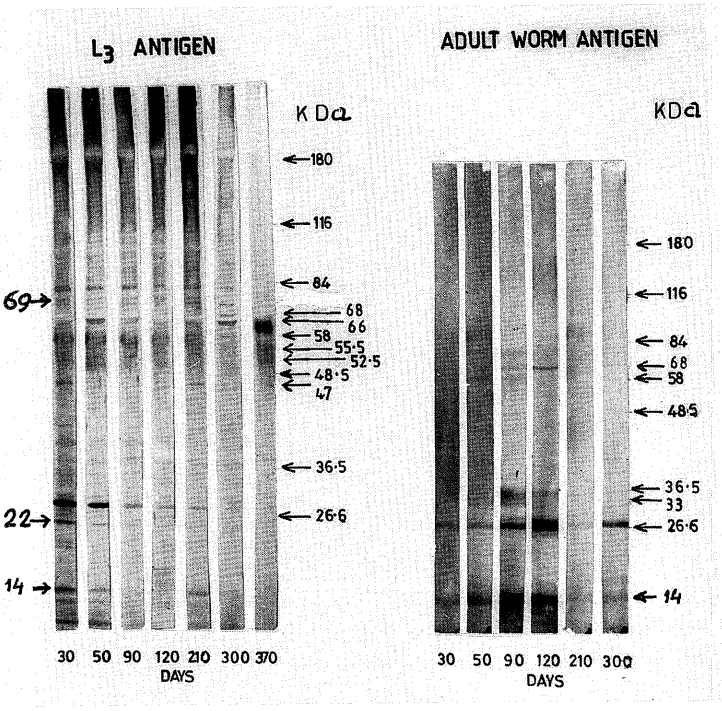

Fig. 2. Immunoperoxidase staining of western blot of *A. viteae* antigens by infected mastomys sera of different durations
a. infective larval antigen; b. adult worm antigen.

Microfilarial antigen

A large number of mf antigens were detected to be immunoreactive during patent stage but a few were functional during latency. Around 300 days serum did not react with any of the fractionated protein bands in blots. Some of the molecules which were identified by the patent serum appeared functional during prepatency also. A double band (84 kDa) was specifically identified during prepatency. Similarly 54 kDa strongly reacted with prepatent but faintly with patent serum and unreactive with latent sera samples. Low Mr functional molecules during early or late patency e.g. 21, 14 and 9 kDa were, however, dominantly recognized and a molecule in 26 kDa region appeared visible in reaction with serum from 120 day's old patent infection (Fig. 3a).

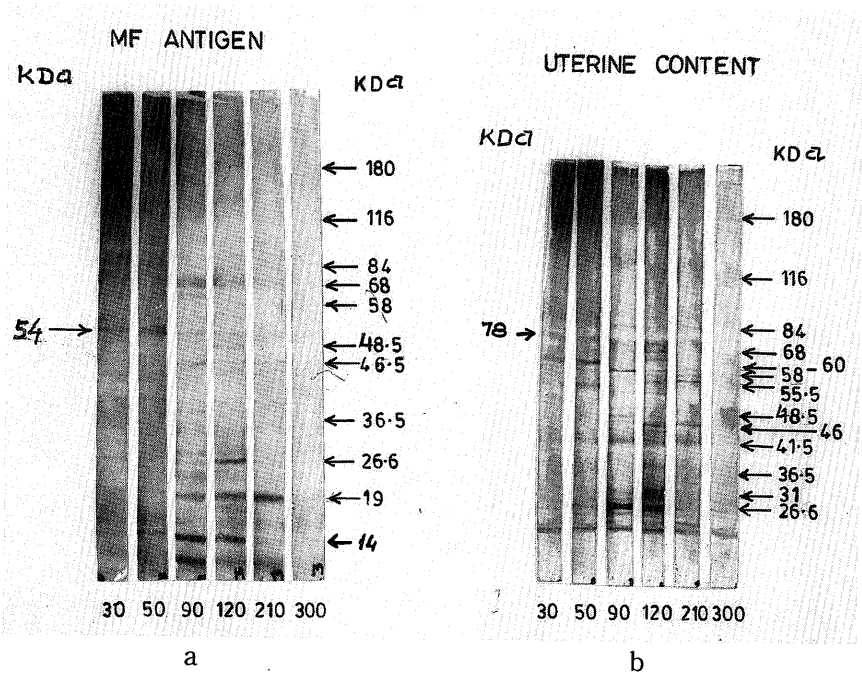

Fig. 3. Immunoperoxidase staining of western blot of *A. viteae* antigens by infected mastomys sera of different durations
a. microfilarial antigen; b. uterine contents.

Uterine antigens

Uterine antigens consisting of uterine wall, uterine fluid, developing eggs and microfilariae were found to possess many immunoreactive components, a number of which reacted with antibodies in sera of all stages of infection. Although highest number of bands were identified by patent (microfilaraemic) serum, a few of them also reacted with prepatent or latent serum. A triplet of bands in 58 kDa region was recognized only by sera of 120 and 210 days infected animals. Antigens of 41.5, 36.5, 31 and 36.6kDa were functional during patency only including few components of 180 kDa and >116 kDa. The 66 kDa component was identified at prepatency only while 78 and 68 kDa at prepatent and patent stages of infection (Fig. 3b).

Sharing of antigen molecules between A. viteae and human filariids

Many antigens of different life-stages of *A. viteae* cross-reacted with human filarial sera. Strongly reactive molecules mostly belonged to low Mr region. Different categories of human filarial sera (pooled) belong to microfilaraemic, symptomatic and endemic normal groups reacted with most of the *A. viteae* antigens in blots. However, most intense reaction was observed with mf positive serum. It was also interesting to observe that many molecules which were recognized by homologous mastomys serum also reacted with human filarial

antibodies. Notable amongst these are 200 kDa, 93, 84, 66, 58, 43, 41, 36.5, 33, and 28 kDa which were shared amongst both *A. viteae* and *W. bancrofti.* Amongst these 200, 66, 43, 41, 36. 5 and 28 kDa reacted with all categories of human filarial sera (Fig. 4).

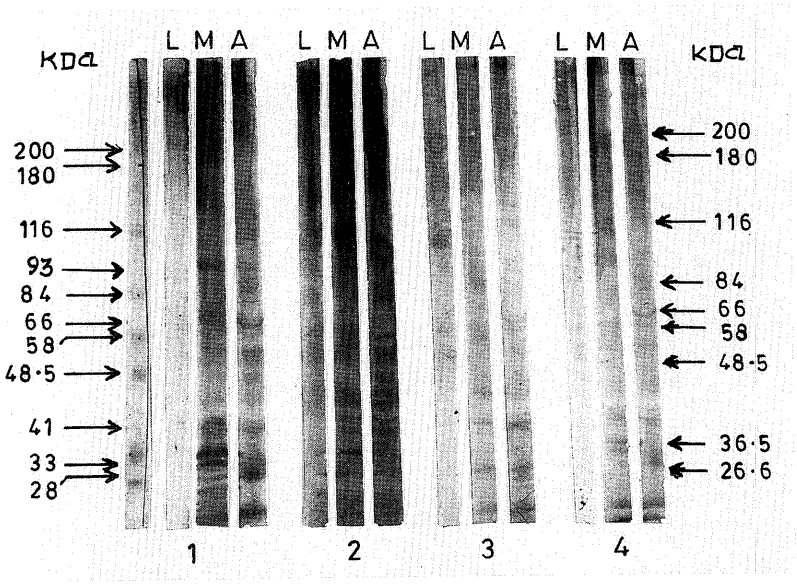

Fig. 4. Immunoperoxidase staining of western blot of *A. viteae* antigens by 1. infected mastomys sera; 2. microfilaraemic human sera; 3. symptomatic human sera; 4. endemic normal human sera. L, M, A, denote infective larval, microfilarial and adult worm antigens respectively.

Discussion

Evidences amply suggest that host reacts immunologically to different life-stages of filarial parasites (Lucius *et al.,* 1986; Lal and Ottesen, 1988; Singh *et al* 1987, 1988). Nevertheless, the constraints of immunopathogenecity of several parasite molecules has necessitated to differentiate them from those responsible for development of immunity against filarial infection. Amongst various life-forms occurring in vertebrate host, mf and adults stay for prolonged periods and most of the immune-reactions are expected to be directed towards several of their antigenic molecules. L_3 although have a brief spell of existence, being the first life-form to enter into the vertebrate host, evoke specific immune-response of host initially.

In the present study antigen recognition pattern of sera of mastomys at different durations of infection was detected to be qualitatively different when antigens derived from adult, mf, L_3 or uterine contents were used. The study indicates that there are common as well as specific antigens when each life-stage of the parasite is analysed. Whereas immune-effects of certain antigens are long lasting and elicit responses during the whole course of infection, yet a few molecules belonging to a particular developmental stage persist till the life-span of that particular life form and antibody response to them comes down or disappear after the

elimination of those antigenic stimuli. Antigens of Mr 26.6, 68, 14 kDa besides few others were present in all the life-stages of *A. viteae* and recognized by sera of different duration of infection. The 26.6 kDa although reacted with sera throughout the course of infection but patent sera does so the most. Nevertheless, L_3 derived 26.6 kDa has stronger affinity for prepatent serum. Another, immunoreactive molecule ($\sim$ 84 kDa) was present in L_3, mf and uterine contents but not adult filariids. Largest number of L_3 derived molecules are observed to be functional and reacted with infected serum. Lucius and his coworkers (1986, 1987) reported strong recognition of L_3 antigens of *A. viteae* (25, 26, 27 kDa in adults; 63 kDa in mf and adults; 45, 68 and 165 kDa in L_3 and 45 kDa in L_3, mf and adults) by antisera and linked 63 kDa of mf of *A. viteae* with clearance of mf from blood in rodents. 46.5, 68 and few lower Mr antigens of mf and a triplet (55.5, 58, 60 kDa) and 46 kDa of uterine contents might be important in the disappearance of microfilaraemia as they remain persisted during latency. Earlier Fletcher *et al* (1986) working with *B. malayi* observed recognition of a triplet (61, 64 and 67 kDa) of bands in mf antigens in cats which suddently became amicrofilaraemic. 66−68 kDa molecule of *A. viteae* and *Brugia malayi* adults have been shown by us to react with human *W. bancrofti* sera (unpublished) which shows the practical applicability of this molecule of heterologous *A. viteae* parasite in immunodiagnosis or immunoprophylaxis of human lymphatic filariasis. A number of molecules have been identified by several workers in different filariids and host species which have been ascribed to play a protective role. These are 18 kDa surface antigen of mf and 30 kDa of adult *O. volvulus* (Dinman and Scott, 1990; Wandje *et al,* 1990), 29−31 kDa surface antigen of *Loa loa* (Egwang *et al,* 1988). 63 kDa of *B. malayi* adults (Perrine *et al,* 1988), 25 kDa of surface antigen of *B. malayi* mf (Kazura *et al,* 1986) and 43 kDa molecule of L_3 of *Brugia malayi* (Freedman *et al,* 1989). The identification of these molecules in animal and human filariids opens up the possibilities for their further experimental evaluation and molecular cloning of the selected ones for production of immunogen (s).

Antigens recognized by a great population of patients from different geographical origins and with different clinical forms of infection could be of particular interest. The use of heterologous antigens with identification and purification of those molecules shared by the human filariid, *W. bancrofti* would eliminate the bottlenecks in the availability of parasite material in required amount. A comparison of antigen recognition pattern of animals with defined stage and duration of infection with similar degree of L_3 exposure under identical conditions (which is not practically feasible in human subjects) makes it possible to identify antigens in L_3, mf or adults for prophylactic or diagnostic use. Cross-reaction between *A. viteae* antigen and *W. bancrofti* human sera led to identification of a few common molecules especially 200 kDa of L_3, mf adults, 66 kDa of adults, 41 and 28 kDa of mf and adults, 36.5 kDa of mf molecules. This sharing of antigens of *A. viteae* by lymphatic human filariid, *W. bancrofti* demonstrates the usefulness of antigens of rodent filariid *A. viteae* in immunodiagnosis or in evolving strategies for vaccination against human filariasis. Interestingly 66, 41, 40 and 36.5 kDa were also expressed in *A. viteae*/mastomys serum, it can be inferred that the results obtained in *A. viteae*/mastomys model may possibly be translated in human filarial subjects. Since 66 kDa molecule of adult is dominantly recognized by symptomatic and endemic normal sera, this molecule might have a protective function.

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