cross-binding of MAbs with heterologous types of FMDV was observed, so far cross-neutralization of MAbs with heterologous types has not been reported.

MAbs III/A4, III A6 and I B4 reacted with separated VP1 of A22, whereas MAb I, D4 did not react with any of the separated VPs (Table 5). An interesting observation was that MAb III/A4 bound to separated VP3 in addition to VP1. These results proved the presence of at least three distinct neutralization epitopes on the surface of FMDV subtype A22. One of them is probably conformation-dependent present on the intact 146S particle and best defined by MAb I/D4, while the second was perhaps conformation-independent present on separated VP1 recognized by the other MAbs III/A6 and I/B4. The binding of MAb III/A4 with two different isolated viral proteins may suggest that a particular epitope or at least part of the epitope sequence is repeated on both VP1 and VP3. It is thus possible that the epitope recognized by this MAb III/A4 is different from the other two epitopes defined above. This finding however needs to be further confirmed by adsorption studies as well as further recloning.

Subunit vaccines consisting of FMDV type A VP1-specific immunodominant domain have been effective in protecting cattle and pigs against challenge infection[27,28]. This is presumably due to the generation of antibody specific to the neutralization epitopes on the VP1 structure. However, inducing protective immunity in these animals with a VP1 preparation derived from type O virus was not successful[29]. The latter observation underscores the need of development of strategies for synthesizing conformational as well as sequential epitopes as protective immunogens of FMDV.

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Acanthocheilonema viteae in *Mastomys natalensis*: Evaluation of efficacy of microfilarial vaccine

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The efficacy of microfilarial antigens derived from *Acanthocheilonema viteae* has been evaluated in normal as well as exposed animals simulating human population in filaria-endemic area. The crude antigen (CAg) or one of its Sephadex fractions (Fr. I) offered only microfilarial-stage specific resistance against challenge made with either infective larvae, microfilariae or adult parasites. Subsequent study with CAg showed better protection, including significant effect on adult parasite population, if the recipients were already carrying a prepatent infection, or displaying patent microfilaraemia or even

challenged with a second exposure. SDS–PAGE and Western blot analysis of microfilarial antigen revealed certain low-molecular-weight antigens to be responsible in the development of resistance against infection. The resistant sera that induced strong *in vitro* cellular adherence and cytotoxicity to microfilariae, transferred resistance to naive animals on passive transfer.

CONTROL of filariasis with chemotherapy alone appears inadequate. There is need for development of suitable immunoprophylactic agent as an adjunct to chemotherapeutic measures. The existing information indicates that microfilariae are quite immunogenic, displaying acquired resistance against animal and human filarial infections[1–3]. Moreover control of microfilaraemia with microfilarial antigen would lead to blocking of transmission of the infection. Thus it is worthwhile elucidating the efficacy of microfilarial antigen not only against microfilariae but also against other stages, specially adult filariids. The other aspect of immunoprophylactic measure using a vaccine that needs further clarification is the possible outcome of application of such an agent in an endemic area where subjects with different stati of filarial infection would be present.

The present study therefore aims at evaluation of antigens derived from microfilariae of *Acanthocheilonema viteae* in *Mastomys natalensis* under healthy condition as well as under prepatent or patent stages of homologous infection-simulating conditions prevailing in a filaria-endemic area.

Six-week-old male mastomys were infected with 50 infective larvae (L_3) subcutaneously[4]. Microfilariae were isolated from infected blood by passing it through membrane filter (5.0 μ)[4], washed and finally suspended in phosphate-buffered saline (PBS) (pH 7.2). Microfilariae were sonicated at 10 kcs for 10 min (Soniprep, MSE) and centrifuged at 10,000 g for 30 min at 4°C. The supernatant was aspirated and protein estimated[5].

The method of Neilson[6] for gel filtration was broadly followed. Briefly, 1.5 ml (10 mg protein) of soluble antigen was loaded onto Sephadex G-200 column (1.5 × 35 cm) previously equilibrated with PBS, 0.01 M phosphate buffer pH 7.4, 0.15 M NaCl. Fractions (3 ml) were collected at the rate of 25 ml h^{-1}. Fractions of each peak were pooled, dialysed and lyophilized, and protein content was measured[5].

Immunization schedule is given in Table 1. First dose of antigen was given along with Freund's complete adjuvant (FCA). After immunization naive animals were challenged with L_3, microfilariae or adult parasites as shown in the table.

Tail blood (5 mm^3) under nembutal anaesthesia was taken from each animal for assessment of microfilaraemia. In L_3-challenged animals, microfilarial examination was done on day 60 and thereafter every fortnight till day 120 when the animals were autopsied to assess the recovery of adult parasites. In case of microfilariae or adult worm challenge, blood for microfilariae was first examined on days 4 and 7, thereafter every week till day 42.

Sera and spleen cells from resistant animals were passively transferred to naive animals. One group received 3×10^8 cells and 0.5 ml serum from resistant animals and another group was administered with cells and serum from normal mastomys. Two other groups received cells or serum (immune) alone. Microfilariae were examined in the blood of all animals following challenge with live microfilariae (i.v).

Crude and fractionated antigens were run in 7–15% (w/v) gradient slab gels at 25 mA for 4–6 h following broadly the technique of Laemmli[7] with modifications[8]. Electrophoretically separated antigens were transferred to nitrocellulose (NC) sheet[9]. NC strips were incubated with respective immune sera and reactions were visualized by the addition of 3,3′-diaminobenzidine (Sigma) after reincubation of strips with second antibody[10] (goat antimouse IgG + IgM horse radish peroxidase conjugated; Medac, Hamburg).

Twentyfour mastomys were infected for 30 days, divided into six groups and immunized with crude antigen. Details of immunization are given in Table 1.

Blood examination for microfilariae was first done on day 60 after first L_3 exposure, then on day 75 and continued till day 75 after 2nd L_3 exposure. All the animals were killed on day 90 and adult worm burden assessed.

Microfilaraemic mastomys (75–90-days-old infection) were immunized with crude antigen. One group received

Table 1. Plan of immunization

Animal groups*	Immunogen**	Challenge (subcutaneous route)
Naive animals		
Antigen	Crude	50 L_3/0.2 million microfilariae
		5 ♂ + 11 ♀ worms
Antigen	Fr. I	"
Antigen	Fr. II	"
Antigen	Fr. III	"
FCA	—	"
PBS	—	"
Prepatent animals		
Antigen	Crude	—
Antigen	Crude	50 L_3
PBS	—	50 L_3
PBS	-	—
Antigen	Crude without FCA	—
FCA	-	—
Patent animals		
Antigen	Crude	-
Antigen	Crude without FCA	-
FCA		-

*Four to ten animals were used in each experiment
**Three inoculations of antigen at fortnightly interval at 10, 25 and 50 μg except crude one, which was administered at 25, 50 and 75 μg

antigen in FCA, second group only antigen and the third FCA only (Table 1). Microfilaraemia was assessed in the tail blood just before antigen administration and on days 4 and 7, thereafter every week till day 49. Animals were killed on day 50 and adult worm recovery assessed.

Sephadex G-200 filtration yielded three peaks. Fr. I consisting of 21 ml volume had total protein content of 2.65 mg. Fr. II containing 12 ml volume had 1.666 mg protein while Fr. III consisting of 15 ml volume had 1.533 mg total protein content (data not shown).

Microfilaraemia was significantly suppressed in challenged (L_3) animals immunized with either crude antigen ($P<0.001$) or Fr. I ($P<0.01$) (Figure 1) without affecting adult worm burden. Even when challenge was made with microfilariae or adult parasites, microfilariae density was markedly low in crude antigen, Fr. I or Fr. II immunized group (figure not shown).

Sera of resistant animals conferred protection against microfilariae on passive transfer and caused strong *in vitro* adherence of cells to microfilariae (data not shown).

Antigen caused significant suppression of microfilaracmia ($P<0.001$) when administered along with FCA (Figure 2). Adult worm recovery was also reduced ($P<0.05$) (Figure 3). The effect was less pronounced when antigen was given without adjuvant. Such animals even if reinfected (group 2) exhibited lower worm recovery ($P<0.01$) as also microfilaraemia ($P<0.001$).

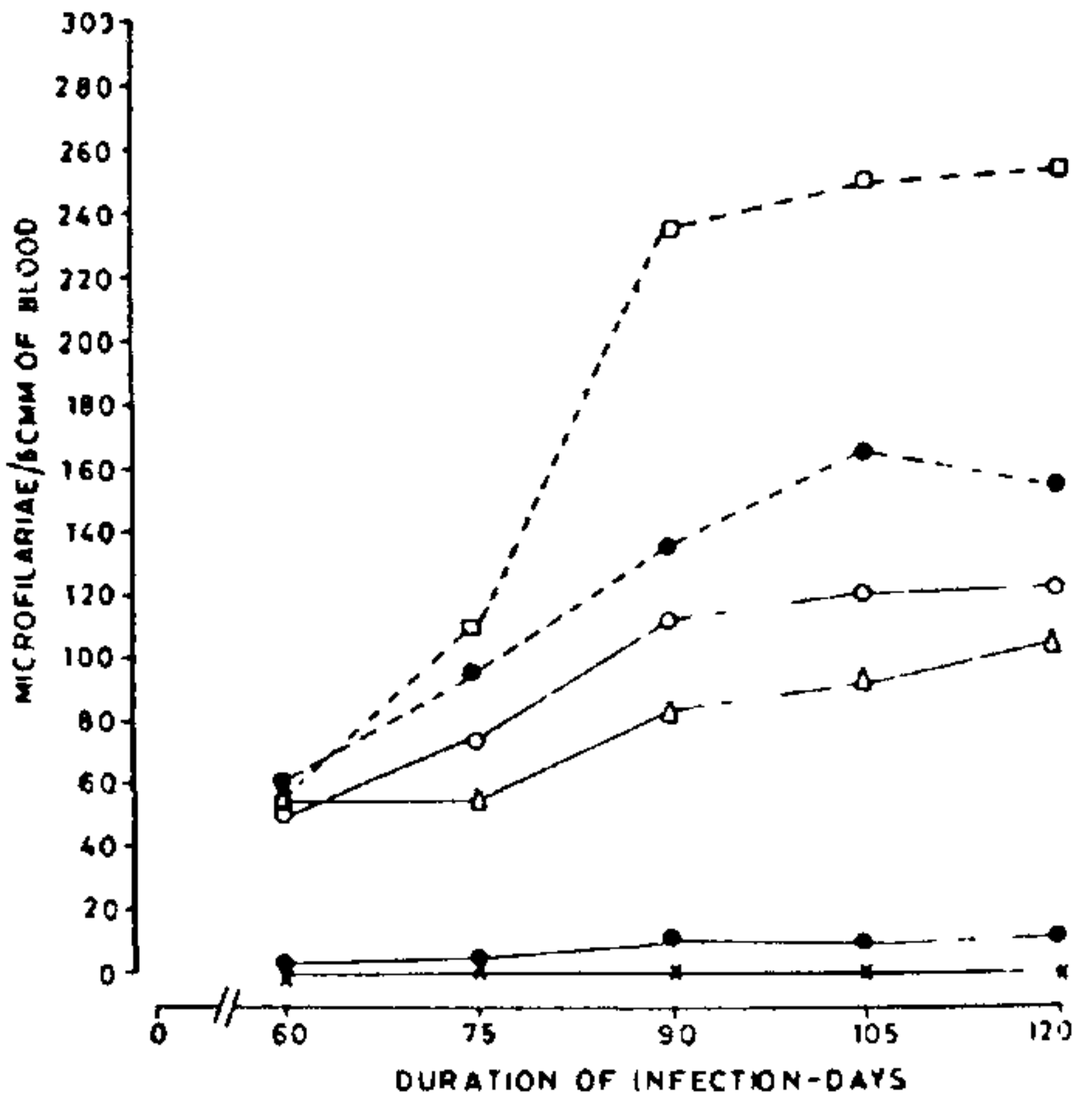

Figure 1. Microfilaraemia in animals immunized with crude and fractionated microfilarial antigen following challenge with L_3. ●——● Crude antigen, ×——× Fr I, ○——○ Fr II, △——△ Fr. III, □——□ FCA control, ●------● PBS control

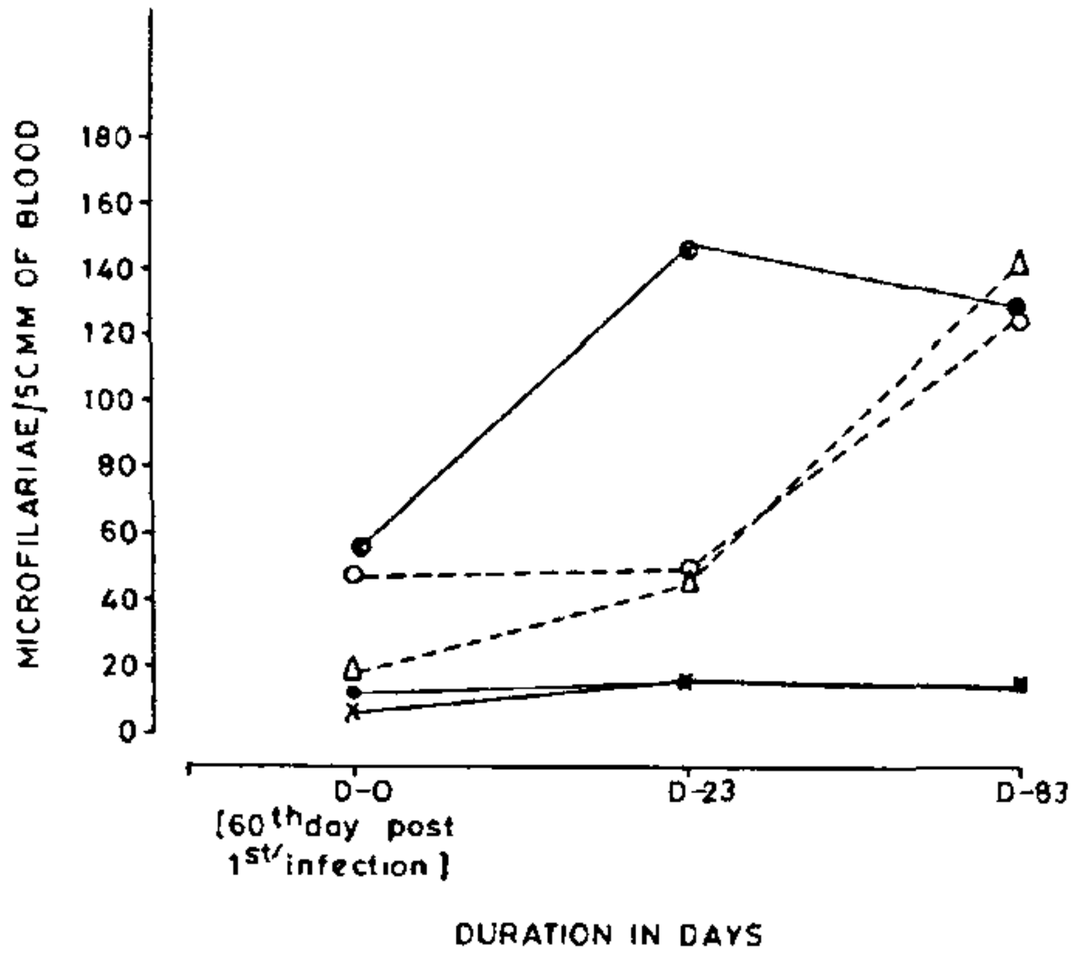

Figure 2. Microfilaraemia in animals immunized with crude microfilarial antigen at prepatency. ●——● Group 1 (infection-immunization), ×——× group 2 (infection-immunization-infection), ○------○ group 3 (infection-infection), △- - - - - △ group 4 (infection), ⊗——⊗ group 5 (infection-immunization) (antigen without FCA).

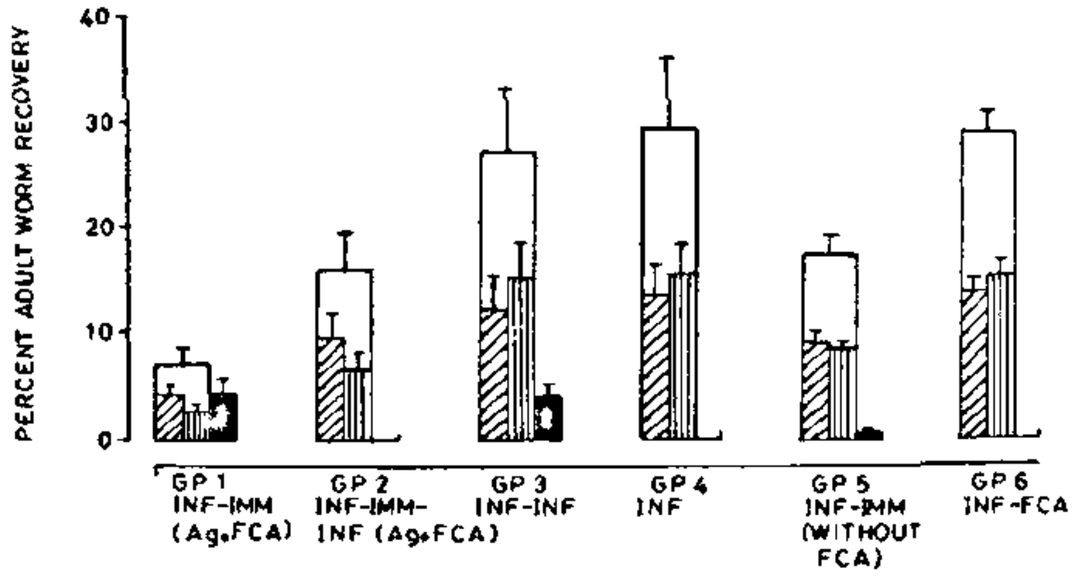

Figure 3. Adult worm recovery from prepatent animals immunized with crude microfilarial antigen. ▨ Male worms, ▥ female worms, □ total worms, ■ nodules.

Worm recovery was comparable in singly (27.5%) and doubly (29.5%) infected mastomys.

Significant suppression of microfilaraemia was also observed when antigen was injected into microfilaraemic animals (Figure 4). The effect was short-lived and recurrence occurred around day 28. Antigen without FCA had almost similar effect (data not shown). There was also decrease in adult worm burden ($P<0.01$) with enhanced formation of nodules (Figure 5).

SDS–PAGE analysis of the three fractions revealed a number of protein bands which, on transfer to nitrocellulose, revealed similar recognition pattern in Fr. I and II in reaction with respective immune serum. Immunized sera identified one band just above 26.6 kD

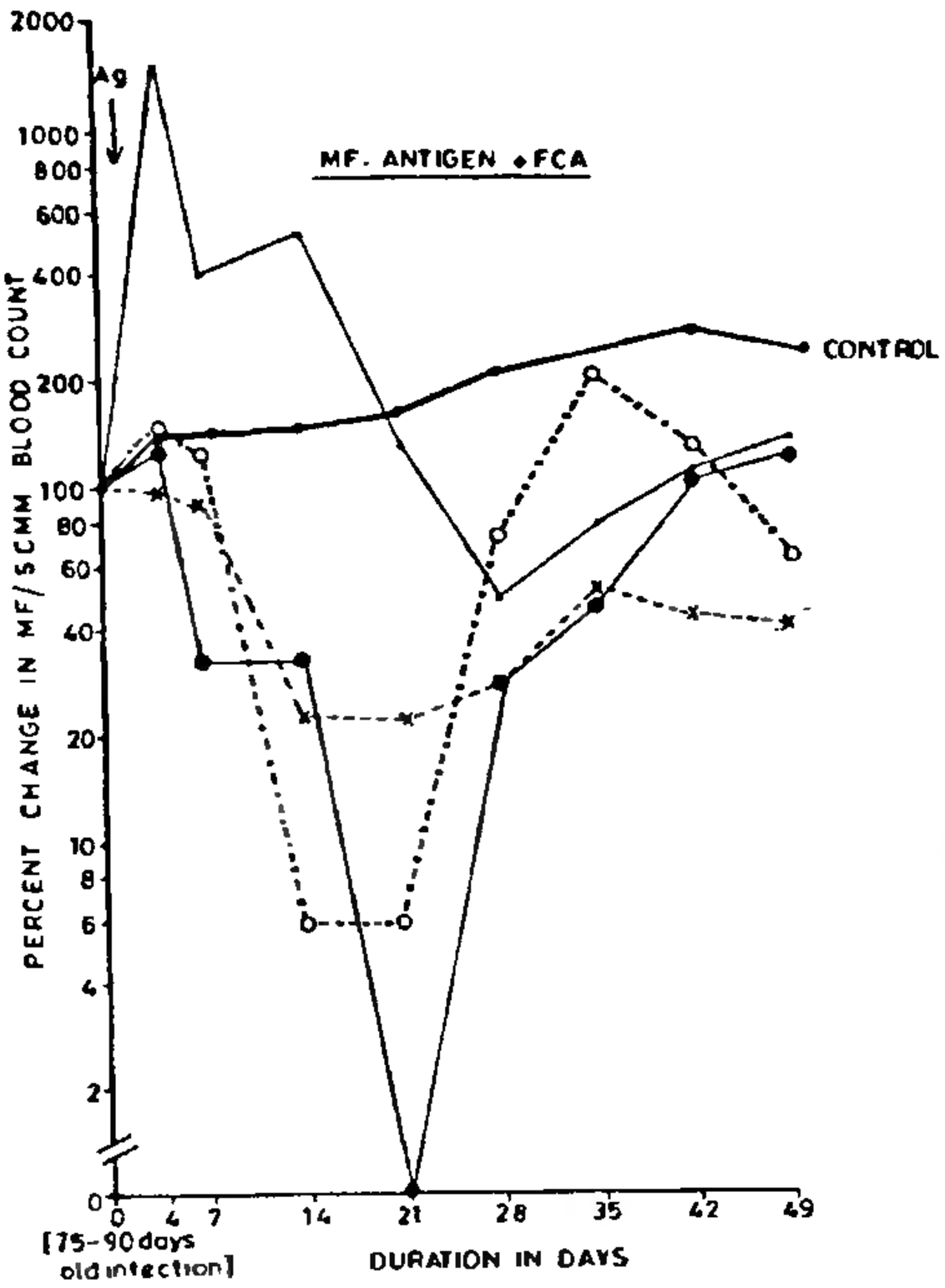

Figure 4 Course of microfilaraemia in patent animals following immunization with crude microfilarial products along with FCA. Data of four individual animals is shown along with the control.

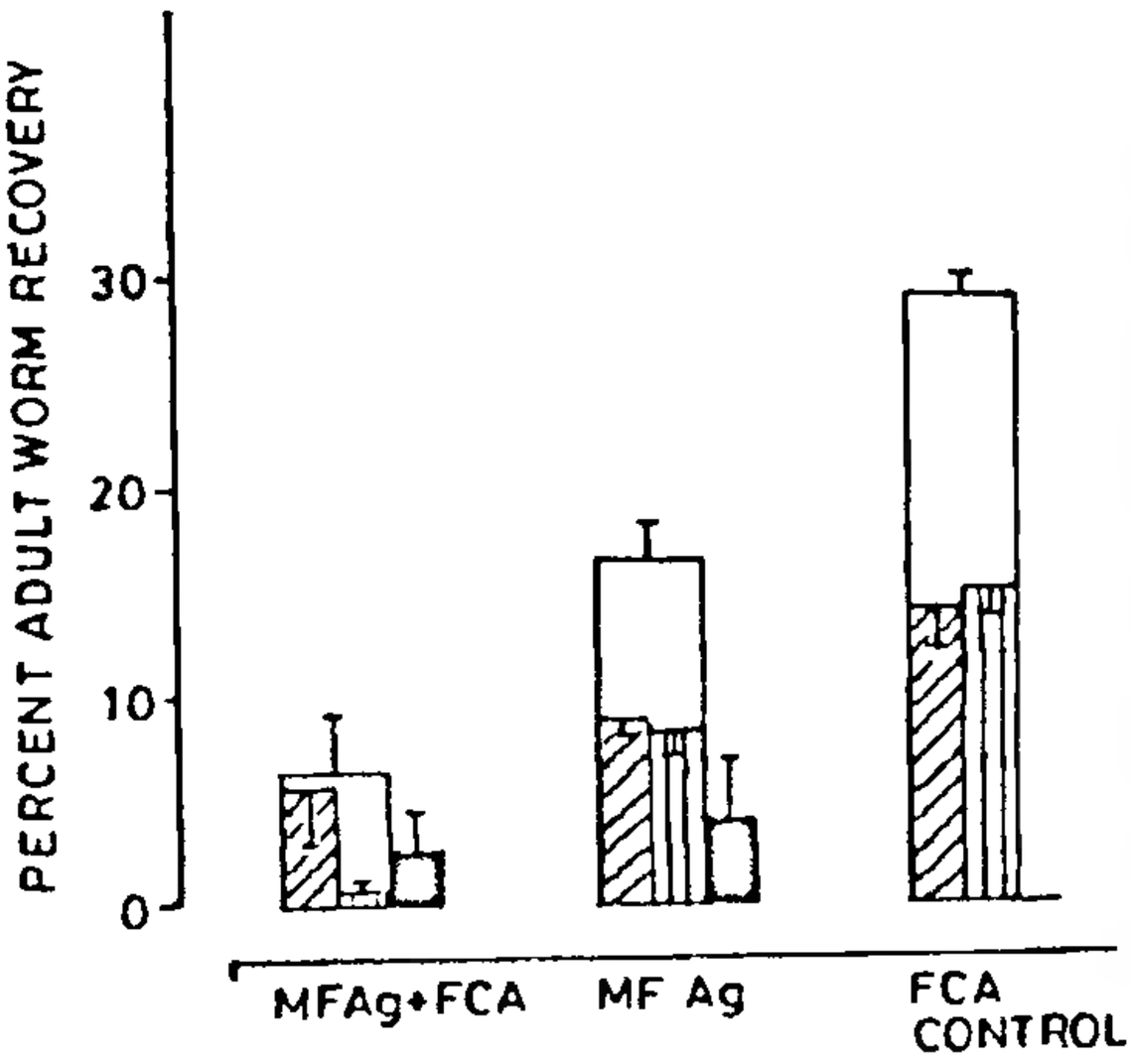

Figure 5. Adult worm recovery from patent mastomys immunized with crude microfilarial products ▨ Male worms, ▥ female worms, ▤ total worms, ■ nodules, (T) indicate mean ± SE.

region, three bands with low molecular weight of approximately 19, 16 and 12 kD, which were not recognized by Fr. III immunized serum (Figure 6).

Control of microfilaraemia with immunoprophylactic measures is an important means in blocking the transmission cycle of filariasis. The method is more pertinent and logical because strong acquired resistance occurs against this very life-stage of filarial parasites both in humans and in laboratory animals[11–16]. Earlier study with live microfilariae yielded good results[1], nevertheless there is always an inherent difficulty of using live parasites. Though subsequent evaluation of crude microfilarial products[2,3] indicated good protective potential, protection was directed only against microfilarial stage. Though the use of microfilarial products and their fractions as immunogen principally affected the development and course of microfilaraemia, interestingly it also had an effect on survival of adult

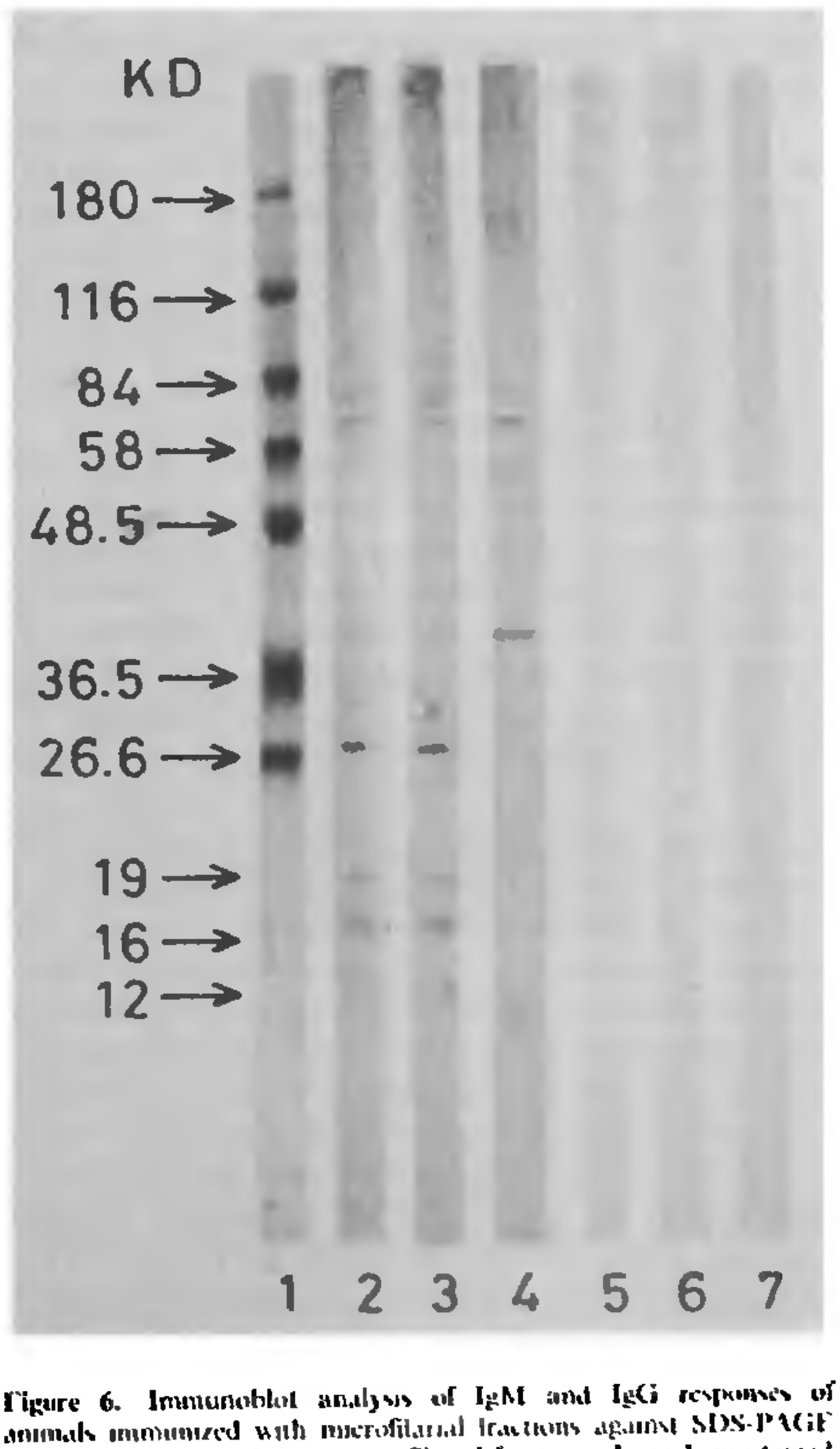

Figure 6. Immunoblot analysis of IgM and IgG responses of animals immunized with microfilarial fractions against SDS-PAGE separated proteins of three microfilarial fractions. Lane 1, prestained marker; lane 2, Fr. I, lane 3, Fr. II, lane 4, Fr. III, lane 5, 6, 7, the three fractions reacted with normal mastomys serum

parasites and possibly establishment of infective larvae to an extent under certain conditions.

Amongst the four microfilarial products the crude one and its three Sephadex fractions, Fr. I appeared to be the best in imparting protection against microfilaraemia whether challenged by microfilarial transfusion or by transplantation of gravid female worms or L_3 inoculation. In spite of strong protection against microfilaraemia there was no effect on adult parasites either transplanted directly or through L_3 inoculation in naive animals immunized with any of the four immunogens tried. A critical analysis of the data on the effect of immunization in naive animals has revealed that efficacy of Fr. I is comparable to the crude product of microfilariae of *A. viteae* (Figures 2 and 3). The crude microfilarial antigen when used as immunogen in animals with developing infection (incubation stage) caused not only very significant suppression in microfilaraemia but also led to very significant reduction in adult worm recovery. Nevertheless, the immunizing potential of the antigen was considerably boosted when applied along with immunoadjuvant.

An extended study using animals with patent infection also revealed serious effect on the survival of adult parasites. Though no further challenge infection was given to patent animals following immunization due to obvious reasons of involvement of age resistance encountered in mastomys with *A. viteae* infection, the study indicated enhancement of acquired resistance against adult parasites following immunization. The female worms were however significantly affected more in comparison to their male counterparts. Though cross-reactive immunogenic antigens between microfilariae and adult worms have not been studied, the present observation does not rule out such a possibility. The beneficial effect of immunization in subjects already having infection has been observed in humans with leprosy[17,18], where suppressed cell-mediated immune status was revoked.

Analysis of microfilarial antigens indicated similar antigenic pattern between Fr. I and Fr. II while Fr. III showed somewhat different antigenic mosaic. Thus antigen just above 26.6 kD and a few low-molecular-weight antigens identified by Fr. I and Fr. II immunized (resistant) sera possibly indicate their involvement in the development of stage-specific protection. The other antigens as identified between 58 kD and 84 kD in all the three fractions by respective immune sera, however do not appear to be involved in protection. Therefore, antigens (above 26.6 kD and 19 kD, 16 kD and 12 kD) of Fr. I and Fr. II need further exploration. Fletcher *et al.*[16] reported selective recognition of certain antigens (67, 64 and 61 kD) by sera of *B. pahangi*-infected cats which suddenly became amicrofilaraemic and attributed these molecules to be responsible for the development of antimicrofilarial antibodies. Certain microfilarial antigens (between 70 kD and 75 kD) have also been identified by sera of amicrofilaraemic human subjects but their role in amicrofilaraemia has been doubtful as amicrofilaraemia could be due to several reasons (false negatives) in human beings. In addition, various degrees of tolerance and/or suppression of immune reactions to filarial antigens observed in native residents of endemic areas[19,20], also complicate the identification of antigens developing protective immunity in human subjects. The present study indicates direct evidence of identification of certain antigenic components (26.6 kD, 19 kD, 16 kD and 12 kD) of microfilariae of *A. viteae* which were recognized by the sera of resistant animals and thus deserve further investigations on their potentiality as transmission-blocking immunogens.

In conclusion, it may be surmised that though certain antigens of microfilarial origin can impart only stage-specific resistance, their use in already exposed cases may hasten the process of acquired resistance against even adult filariids.

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