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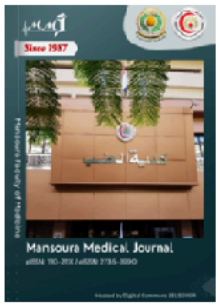

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INDUCTION OF RESISTANCE AGAINST SCHISTOSOMA MANSONI INFECTION USING A PURIFIED 74 KD A SCHISTOSOMA PROTEIN ANTIGEN RECOGNIZED BY PROTECTIVE MONOCLONAL ANTIBODY: HISTOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY

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ABSTRACT

The need for vaccination against schistosomiasis is an achievable goal. In this work the protective and antifibrotic effect of 74 KDa schistosoma protein antigen recognized by protective monoclonal antibody was evaluated histologically and immunohistochemically. Swiss mice were immunized with 50 mg of purified 74KDa antigen without and with Freund's adjuvant. Then mice were infected with 200 S. mansoni cercariae three days after final immunization. The results revealed a protection level of 76.6% and 80% was obtained using the 50mg antigen without and with Freund's adjuvant. Histological examination showed marked decrease of the grnuloma number and size and marked reduction of both collagen and reticular fibers around granulomas of mice liver .Immunohistochemical study revealed decrease of the intensity or even absent of the schistosomal antigen in immunized liver compared to that infected and not immunized.

From this work it can be concluded that in addition of the ability of the 74KDa antigen to mediate protection against schistosoma mansoni infection. It can reduce the granulomatous reaction around eggs lodged in the liver by its ability to modulate the immune system of the infected host. So the protection conferred by the 74

KDa enhanced additional experiments that may lead to improvement of its protective capacity as a candidate vaccine.

INTRODUCTION

Globally, schistosomiasis is one of the most spread parasitic infections affecting humanity. In Egypt bilharziasis is endemic and considered the major ancient public health problem Recent study showed that the prevalence of Schistosoma Mansoni (SM) was ranging between 20 to 40 % in the Nile Delta[1].

The major lesion in schistosomiasis is the granuloma formation that occurs around the living egg trapped in host tissue. There is evidence that granuloma formation is the result of a delayed hypersensitivity reaction initiated by T cell recognition of the egg antigens[2]. The granulomas that form around eggs lodged in the perisinusoidal capillaries impedes hepatic blood flow and the blockage of the portal blood system then induced portal hypertension. The morbidity resulted as a complication of luminal obstruction of hepatic portal venules and this obstruction is largely due to tissue fibrosis that complicated the granulomatous inflammation[3].

Many investigations try to use large scale of chemotherapy for schistosomiasis in an attempt to make resorption of portal fibrosis, since the degradation of fibrous matrix is probably followed by remodeling of the obstructive intrahepatic vascular changes which are primarily responsible for portal hypertension[4] . The granulomatous reaction and the ensuing fibrosis associated with schistosomiasis may be reduced by halting the release of immunogenic compounds from the egg [5]. It was shown that schistosoma eggs, mature in lower numbers after vaccination of mice with immature eggs, and that sera from chronically infected humans are capable of reducing granuloma development[6]. The immediate goal of schistosomiasis vaccine is not only to stop parasite invasion or to block transmission completely but to sufficiently reduce worm burdens and egg production. In addition, it will be attempted also to modulate the granulomatous reaction caused by the presence of eggs in different organ [7].

In previous study done by Attallah et al., (1999) [8] an IgG2a anti- SM mouse monoclonal antibody (mAb) designated as BRL 4 has been produced. This mAb was found to pas-

sively protect Swiss mice against schistosoma infection. This mAb has been identified a protein (antigen) with 74KDa molecular weight in the extract of SM adult worm. This target anigen was prepared [9].

The main target of this work was to evaluate histologically and immunohistochemically the protective and antifibrotic effect of immunization with a purified 74 KDa antigen (recognized by BRL 4 mAb) on the liver of mice infected with schistosoma mansoni.

MATERIAL AND METHODS

Preparation of monoclonal antibody and 74 KDa antigen extracts:

An IgG_{2a} anti-SM mAb designated as BRL4 mAb was developed as described by Attallah et al. [8] .The antigen extract , that react with this mAb, was prepared from the soluble worms, cercariae and soluble eggs according to Da Silva and Ferri[10]. The protein content was determined by Lowry's method[11]. Using polyacrylamide gel electrophoresis the molecular weight of this protein antigen was 74 KDa. The reactivity of the purified target antigen was tested using Western blot and Fast Dot ELISA [12&13]. The 74 KDa protein antigen recognized by BRL4 monoclonal antibody was prepared and stored at —20°C until use.

Active immunization of mice using the 74 KDa protein antigen recognized by BRL4 mAb. Female Swiss mice (10mice/group) were immunized intraperitoneally with 50 mg antigen/mouse dose without and with Freund's adjuvant (volume/volume) (Difco, Detroit, and USA). The antigen was injected three times (at 0, 14 and 21 day). Then immunized mice were infected with 200 SM cercariae three days after final immunization. In the same time normal mice and mice infected with 200 cercariae were injected with phosphate buffer saline (PBS) (PH 7.2) with the same volumes and the same intervals of antigen to serve as negative and positive control groups. Six weeks post infection with 200 S mansoni cercaria all mice were sacrificed and the number of worm burden (oogram) was determined by injection of PBS in portal vein (liver perfusion)[14]. Data were presented as the mean number of worm and standard deviations. Percent of protection against challenge infection was calculated as the reduction in worm burden relative to mice infected with cercaria and received PBS as a

control. The significance of the differences between control and experimental groups was calculated.

Histological study : Liver bipsies of all groups were fixed in neutral buffered formalin and processed to make paraffin blocks. For each animal liver sections were subjected to the following staining: Haematoxylin and eosin (H & E) for determination of granuloma number and size, Masson trichrome for collagen fibers, Gorden and Sweet for reticular fibers and Weigert resorcin fuchsin for elastic fibers (15). In liver sections stained with H&E hepatic granuloma numbers in ten sections from each mouse liver was calculated in definite area of 0.5 cm^2 using an ocular micrometer. Also, the mean size of ten granulomas per section were measured (i.e. 100 granulomas per animal) using an ocular micrometer by measuring the largest diameter and that perpendicular to it(16).

Immunohistochemical study:- for detection of schistosoma antigen in liver sections indirect immunoperoxidase technique was used. Positive reaction seen as brown red colored areas at the site of antigen antibody reaction (17). Negative control was obtained by omitting monoclonal antibody and positive control was obtained from Prof Dr Attallah.

RESULTS

In mice immunized with the 74 KDa antigen either without or with Freund's adjuvant produced significant resistance compared with control group. Using 50mg dose a protection levels of 76.6% without adjuvant and 80% with adjuvant were obtained .

Histological Results . In liver sections of mice infected with 200 S cercariae and stained with H&E great number of granulomas that concentrically arranged around trapped eggs were observed. These granulomas formed of mononuclear leukocytes (mainly eosinophils) and fibroblasts (Fig.A1&2).Also, there was S. pigments in Kupffer cells of blood sinusoids (Fig.A3 &4). In mice immunized with 74 KDa antigen then infected with S. cercariae there was marked decrease of both periovular granulomas number and size and decrease of S. pigments (Fig .A 5 &6) .The 50mg dose of antigen caused reduction of granulomas number up to 51.5% and size to 29.4% ($P<0.05$) without adjuvant and72.6% and 32.3 % with adjuvant ($P<0.05$).

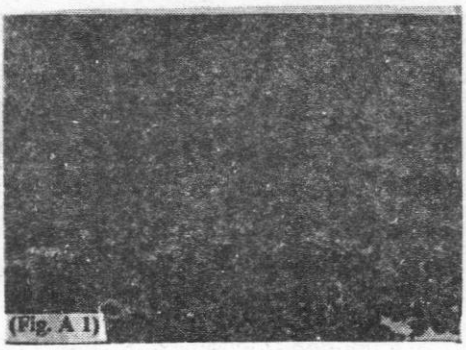
In liver sections stained by Masson trichrome, collagen fibers stained blue. In the normal liver, collagen fibers were present in very small amount in the capsule, interstitial space , the wall of central vein and hepatic artery, portal vein and bile duct of the portal tract (Fig. B1-3). The mice infected with 200 cercariae of SM showed a marked increase of collagen fibers. Collagen fibers of the capsule increase markedly and there was an increase of the interstitial collagen fibers (Fig. B4). Also, collagen fibers become densely packed around the ova and concentrically surrounding central ova in a lamellar fibrosis (Fig.B5-7). When mice received a purified 74KDa then infected with 200 cercariae of SM fibers appeared more or less similar to that of normal liver. The collagen fibers of the capsule decreased (Fig.8). and there was a decrease in the interstitial collagen fibers (Fig.B9). The periovular collagen fibers decreased markedly in the amount, in the density and became loosely arranged and thinner and there was a degradation of collagen fibers (Fig. B 10-12).

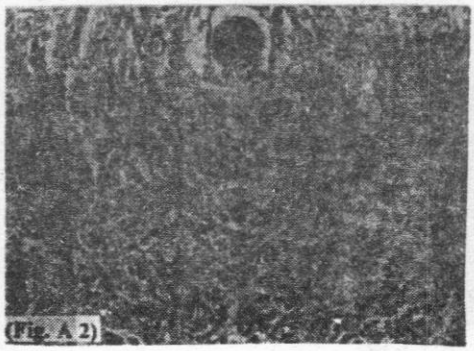
In liver sections stained for reticular fibers these appeared as very fine black fibers. In normal liver,they were present in the capsule ,interstitial space and in the wall of central vein and also in the portal tract (Fig. C1-3). In the infected mice reticular fibers in the capsule were markedly increased (Fig.C4&5). Also, reticular fibers were markedly increased in the periovular granuloma. The majority of them consisted of both collagen and reticular fibers (Fig. C 6&7) and some granulomas formed of reticular fibers only (Fig. C 8&9). In mice immunized with antigen before infection there was decrease of reticular fibers in the capsule (Fig C 10) . Also, the granulomas showed marked reduction of the periovular reticular fibers (Fig. C11 &12).

In normal liver the elastic fibers appeared as brown purple in the wall of central vein (Fig.D 1&2). The same distribution and intensity of elastic fibers were observed in livers of the mice infected with 200 SM cercariae and that of the group immunized with the antigen. However, no elastic fibers were seen in liver granulomas of mice infected with 200 SM cercaria or that received the antigen (Fig. D3).

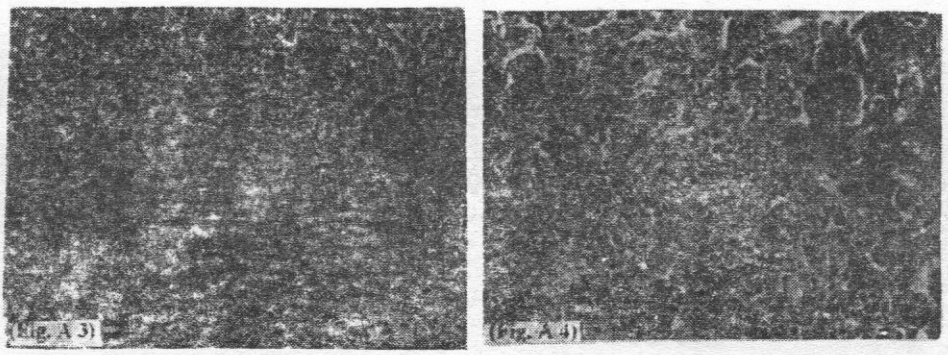
Immunohistochemical results. Liver section of normal control group the schistosome antigen was negative. In liver sections of mice infected with

200 cercariae the schistosomal antigen could be detected strongly on the surface of schistosome egg and in hepatocytes. (Fig.E1-3). Mice immunized with 74 KDa schistosome antigen prior to infection with S. cercaria the antigen showed marked reduction of the intensity and even absent of the schistosomal antigen (Fig.E4-6).

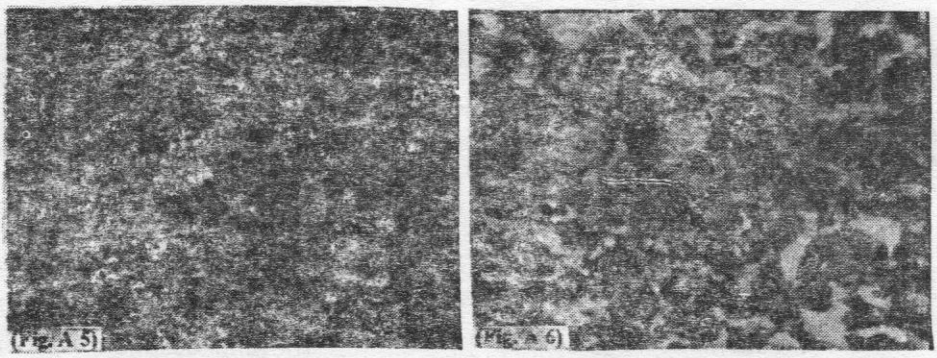




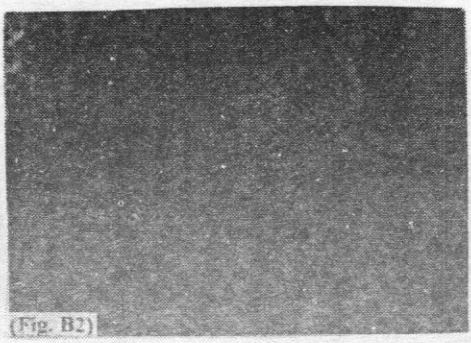
(Fig. A 1&2) : Liver section of mouse infected with 200 S. cercariae showing the granulomas (G) concentrically arranged around eggs (arrows). (H&E stain, X10 &X20).

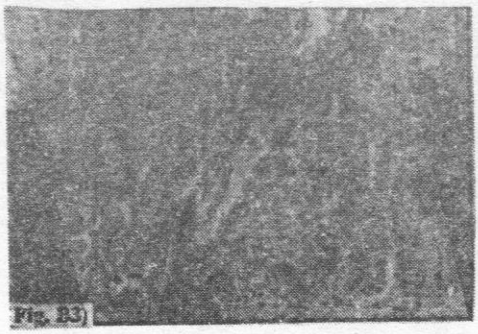


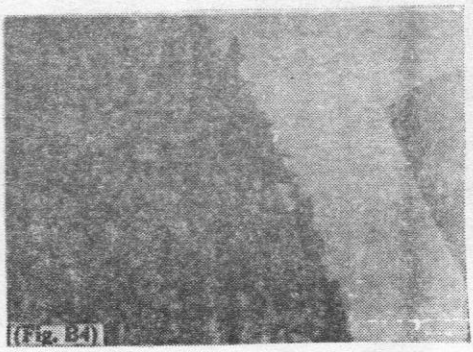
(Fig. A 3&4) : Liver section of mouse infected with 200 S. cercariae showing schistosoma pigments (arrows) in the Kupffer cells of blood sinusoids. (H&E stain X10&X40).



(Fig. A 5&6) : Liver section of mouse immunized with 50 mg of 74KDa antigen then infected with 200 S cercariae showing marked decrease of granuloma (G) number and size (H&E stain X10&X40).

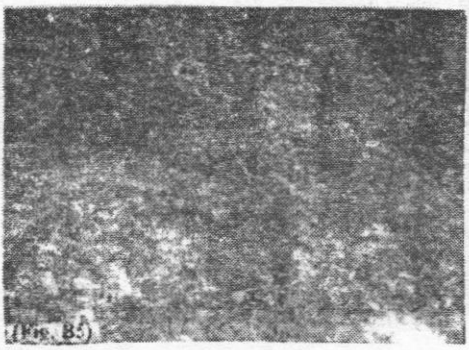


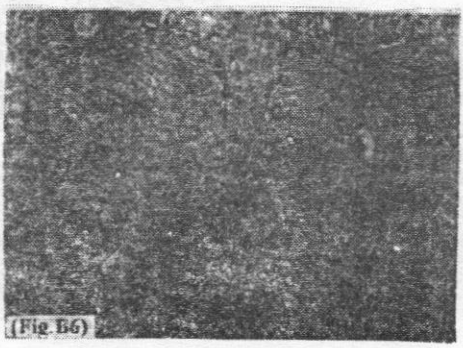


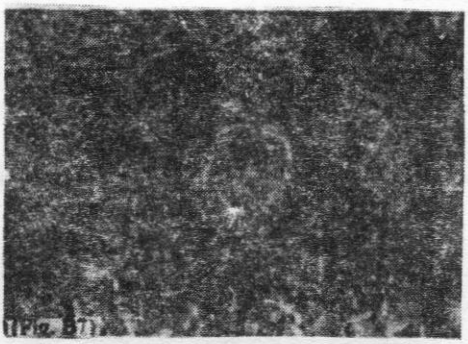


(Fig. B 1-3) : Liver section of normal control mouse showing distribution of collagen fibers in interstitial space, wall of central vein, hepatic artery, portal vein and bile duct of portal tract (Masson trichrome stain, X10&X40 &X40)

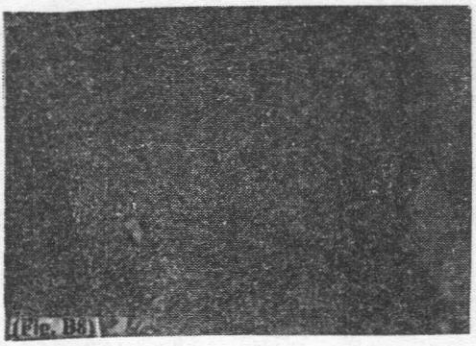
(Fig. B 4) : Liver section of mouse infected with 200 S cercariae showing collagen fibers in the capsule and interstitial space (Masson trichrome stain, X40).



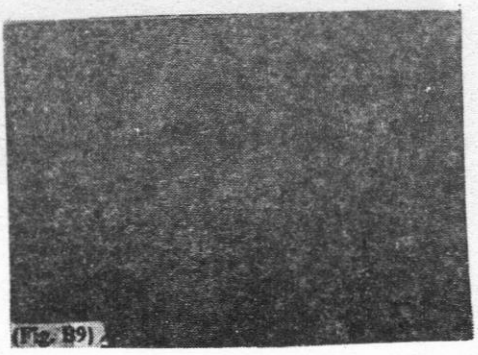




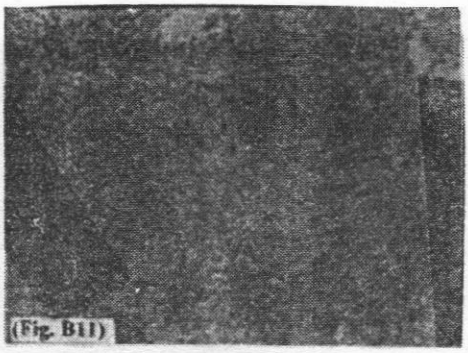
(Fig. B 5-7) : Liver section of mouse infected with 200 S cercariae showing collagen fibers in the granulomas (G) surrounding eggs (E) (Masson trichrome stain, X5& X10 &X40).

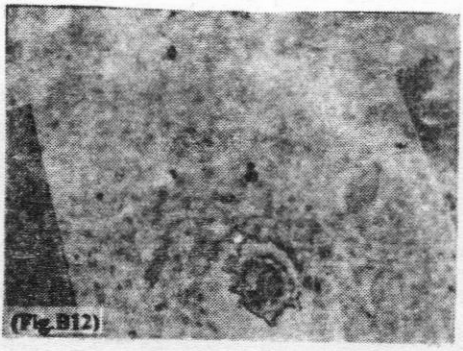


(Fig. B 8) : Liver section of mouse immunized with 50 mg of 74KDa antigen then infected with 200 S. cercariae showing marked decrease of collagen fibers in the capsule (arrow) (Masson trichrome stain X40).

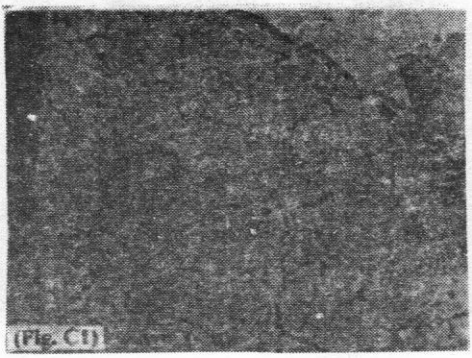


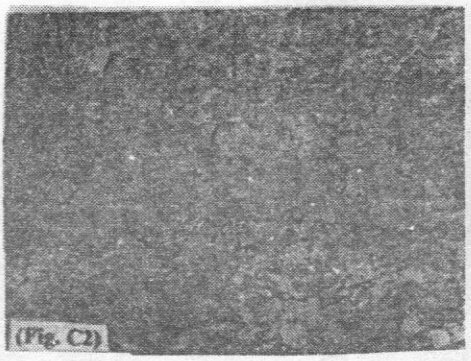
(Fig. B 9) : Liver section of mouse immunized with 50 mg of 74KDa antigen then infected with 200 S cercariae showing marked decrease of collagen fibers in the interstitial space (Masson trichrome stains X40).

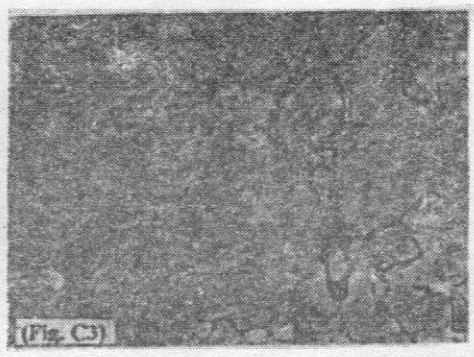




(Fig. B 10-12) : Liver section of mouse immunized with 50 mg of 74KDa antigen then infected with 200 S. cercaria showing marked decrease of collagen fibers in the periovular granulomas (G) (Masson trichrome stain (X5& X10 &X40).

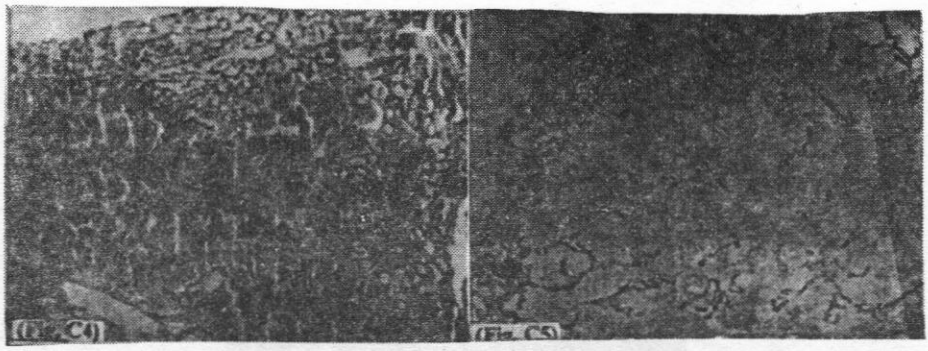




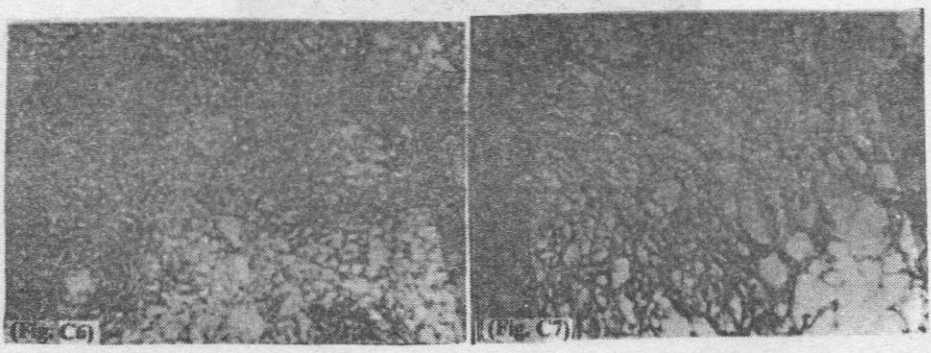


(Fig. C 1-3) : Liver section of normal control mouse showing distribution of reticular fibers in the capsule, interstitial space, wall of centr al vein, hepatic artery, portal vein and bile duct of portal tract

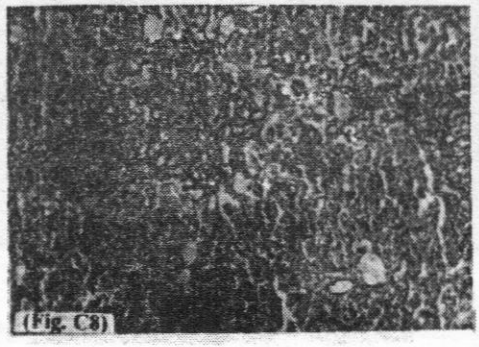
(Gorden & Sweet stain X40)

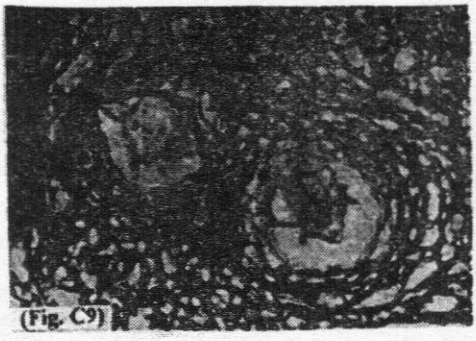


(Fig. C 4&5) : Liver section of mouse infected with 200 S. cercaria showing reticular fibers in the capsule &stroma (Gorden & Sweet stain X10 &X40).



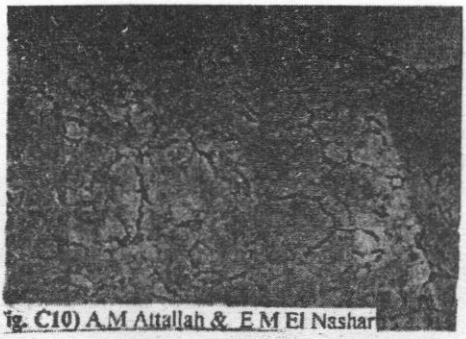
(Fig. C 6&7) : Liver section of mouse infected with 200 S. cercaria showing periovular granulomas formed of both collagen (C) & reticular (R) fibers . (Gorden & Sweet stain X10 &X40).

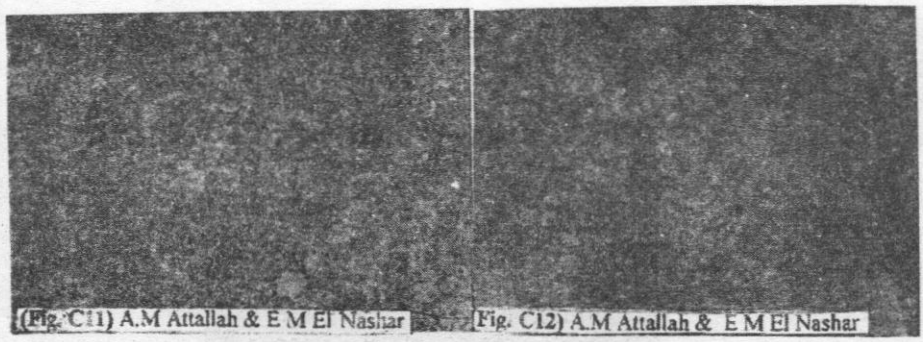




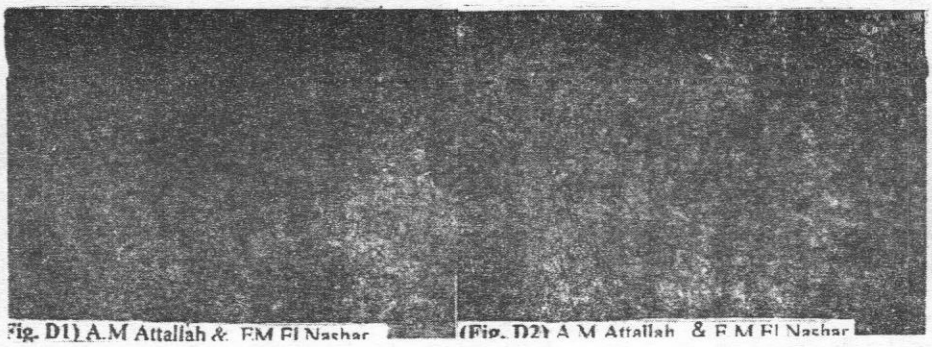
(Fig. C 8&9) : Liver section of mouse infected with 200 S. cercaria showing peri-ovular granulomas (G) formed of reticular (R) fibers only. (Gorden & Sweet stain X10 &X40).

(Fig. C 10) : Liver section of mouse infected with 200 S. cercaria showing reticular fibers in the capsule &stroma (Gorden & Sweet stain X10 &X40).

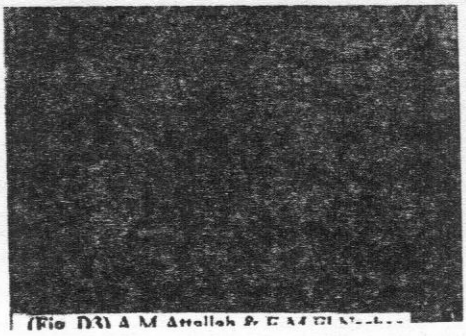




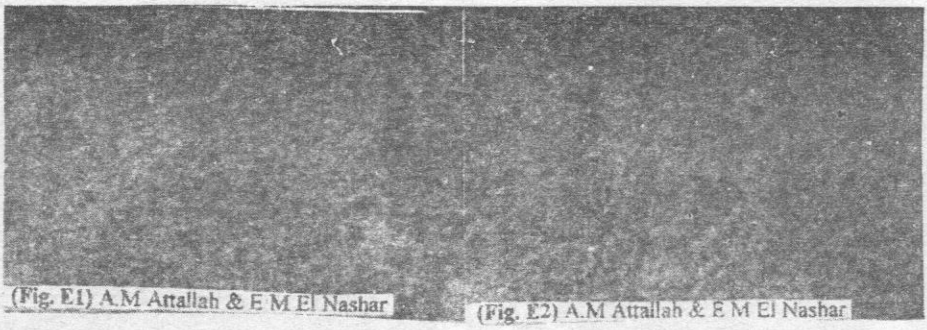
(Fig. C 11&12): Liver section of mouse immunized with 50 mg of 74KDa antigen then infected with 200 S: cercaria showing marked decrease of reticular fibers in the periovular granulomas (Gorden & Sweet stain X10 &X40).

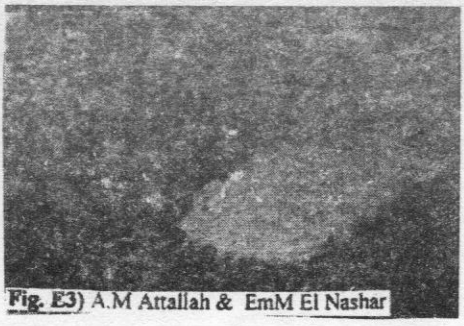


(Fig. D 1&2) : Liver section of normal control mouse showing distribution of elastic fibers (arrows) (Weigert resorcin fuchsin stain X10&X40).



(Fig. D 3): Liver section of mouse infected with 200 S. cercariae showing absence of elastic fibers in periovular granulomas (Weigert resorcin fuchsin stain X10).





(Fig. E 1-3) : Liver section of mouse infected with 200 S. cercaria showing Schistosomal antigens (appeared brown red) on the surface of egg (arrow) and hepatocytes (Indirect immunoperoxidase and counterstained with Mayers Hx, X10 &X40 &X40).

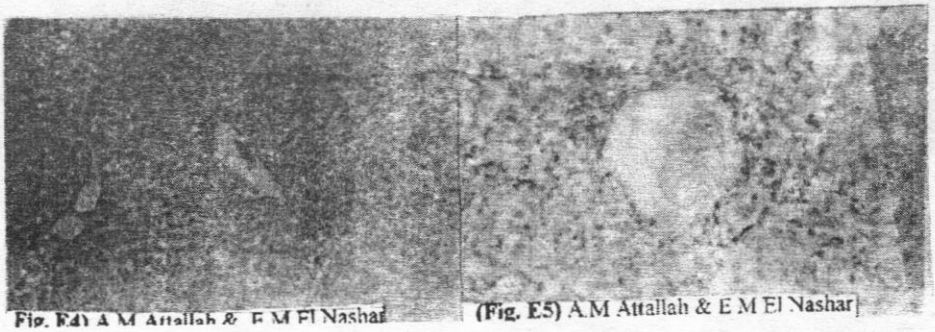
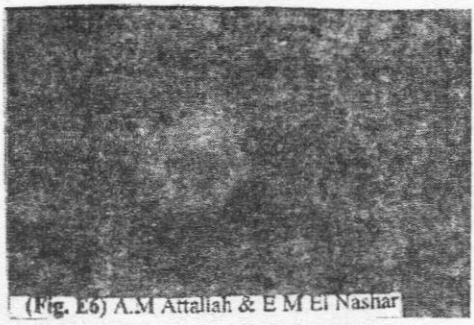


Fig. E4) A.M Attallah & E M El Nashar (Fig. E5) A.M Attallah & E M El Nashar



(Fig. E6) A.M Attallah & E M El Nashar

(Fig. E 4-6) : Liver section of mouse immunized with 50 ug of 74KDa antigen then infected with 200 S. cercaria showing no localization of S. antigens on the surface of S.egg or in hepatocytes. (Indirect immunoperoxidase and counterstained with Mayers Hx ,X10 &X40 &X40).

DISCUSSION

In contrast to most infectious agents, parasites commonly strike a balance with their hosts , which not exclude reinfection. This fact explains the chronic course of the majority of parasitosis and the increased risk for host defense mechanisms causing pathological lesions. For this reason, successful vaccines must target different aspects of the host /parasite relationship and may not necessarily aim at sterile immunity. The immediate goal of schistosomiasis vaccine is not only to stop parasite invasion or to block transmission completely but to sufficiently reduce worm burdens and egg production. In addition, it will be attempted to also modulate the granulomatous reaction caused by the presence of eggs in different organs [18]. Therefore, the identification and characterization of the antigens involved in this immune response are of interest. The development of monoclonal antibodies that adaptively transfer resistance had led to characterization of several potential protective antigens[19].

In previous study Attallah et al.,(1999) [8] produced an IgG2a anti-S M BRL4 mAb that have been shown, using immunohistochemical staining, to react in different intensities and locations with the different developmental stages of SM. Also,it has been shown that mice immunized with 74 KDa antigen there was dose dependant increase of IgG level and hence has an effect on the humoral immune system [20] .

In the present study the effect of active immunization of mice with 50mg of the 74KDa antigen recognized by BRL4 mAb was evaluated histologically and immunohistochemically. A protection level of 76.6% without adjuvant and 80% with adjuvant was obtained. Also, this antigen induced high percent reduction of granuloma number and size. It is of great value as beside protection, if granuloma formation could be prevented or suppressed, the development of severe disease might be averted. On experimental models, many of antigens isolated from parasite forms were able to induce moderate levels of protection [21]. However, these studies did not analyze the induced granulomatous reactivity to egg. So to identify target antigens that provides protective immunity and participates as granulomatous modulating agent in schistosomiasis is one of precious hope. This may indicate the suitability

of the 74KDa antigen as an immunizing agent. The ability of this antigen to modulate the granulomatous reaction in vivo is contingent upon its ability to recognize and binds to epitopes on the surface of the developing schistosome Also, this indicates that the antigen may occupy an essential site on the schistosomula which is vulnerable to antibody mediated damage. This antigen has a dual function as it reduced the worm burden and reduced granuloma numbers and size. In addition cause a marked reduction in the collagen and reticular fibers of granulomas and reduce hepatic fibrosis. Moreover the distribution of collagen and reticular fibers, in livers of mice received 74KDa antigen became more or less similar to that of normal liver. Also, in the hepatic periovular granulomas, these fibers decreased markedly in the amount, in density and became loosely arranged and thinner. This is in agreement with the fact that, the excessive deposition of fibers in several types of lesions is known to undergo resorption when the provocative cause is removed (22).

On the other hand in hepatic periovular schistosomal granulomas produced in mice infected by 200 cercariae no elastic fibers can be detected. This result was in agreement with the results obtained by Junqueria et al., (1986) (23). In contrast Andrade et al., (1992) (24) have found marked hyperplasia of elastic fibers in hepatic periportal fibrosis, in human. The conspicuous absence of elastic fibers from periovular granulomas can be attributed to that elastic fibers in liver disease are related to chronicity of the lesion. The periovular granulomas may not survive the necessary time for excess elastin synthesis to take place. The most likely explanation of the reduction in the granuloma number is that the antigen cause severe damage to essential life functions of the parasite leading to the parasite death and consequently reduction in the number of eggs produced. Also,that there was induction of the host immune responses to the 74 KDa antigen. The cause of marked reduction of liver fibrosis observed may be explained as vaccination with the 74KDa antigen was associated with a state of immunoregulation with an inhibitory effect on cell mediated immunity resulting in the formation of small fibrocellular granulomas. Also, this antigen can alter the expression of granulomatous reaction to the parasite eggs, diminishe the liver cells damage so give

good and consistent protection against challenge infection [25].

In addition immunohistochemical study of the livers to detect schistosomal antigen revealed marked reduction of schistosomal antigen in livers immunized with 74KDa schistosomal antigen. This mean absence of schistosoma antibody in the liver of mice immunized with 74KDa antigen. So we can say that 74KDa antigen may act by reacting with the egg component and could be able to modulate the immune response against schistosoma eggs.

In conclusion the 74KDa target antigen has been characterized as a protein has the ability to protect mice.Also, marked reduction of granuloma number and a considerable change in the intensity of collagen and reticular granuloma fibers in liver and periovular granulomas.In addition absence of schistosoma antigen in the liver were observed in immunized mice. These are indicative of ability of this antigen to modulate the immune system and its suitability as an immunizing agent. This antigen may play a role in the host parasite interplay, as this protective antigen may induce in the host an immune response that lead to severe damage to essential life functions of the parasite. Taken all together, additional subsequent experiments might lead to improvement of the protective capacity of this antigen as an immunogen.

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