MOLECULAR CHARACTERIZATION AND EXPRESSION OF SCHISTOSOME ANTIGENS

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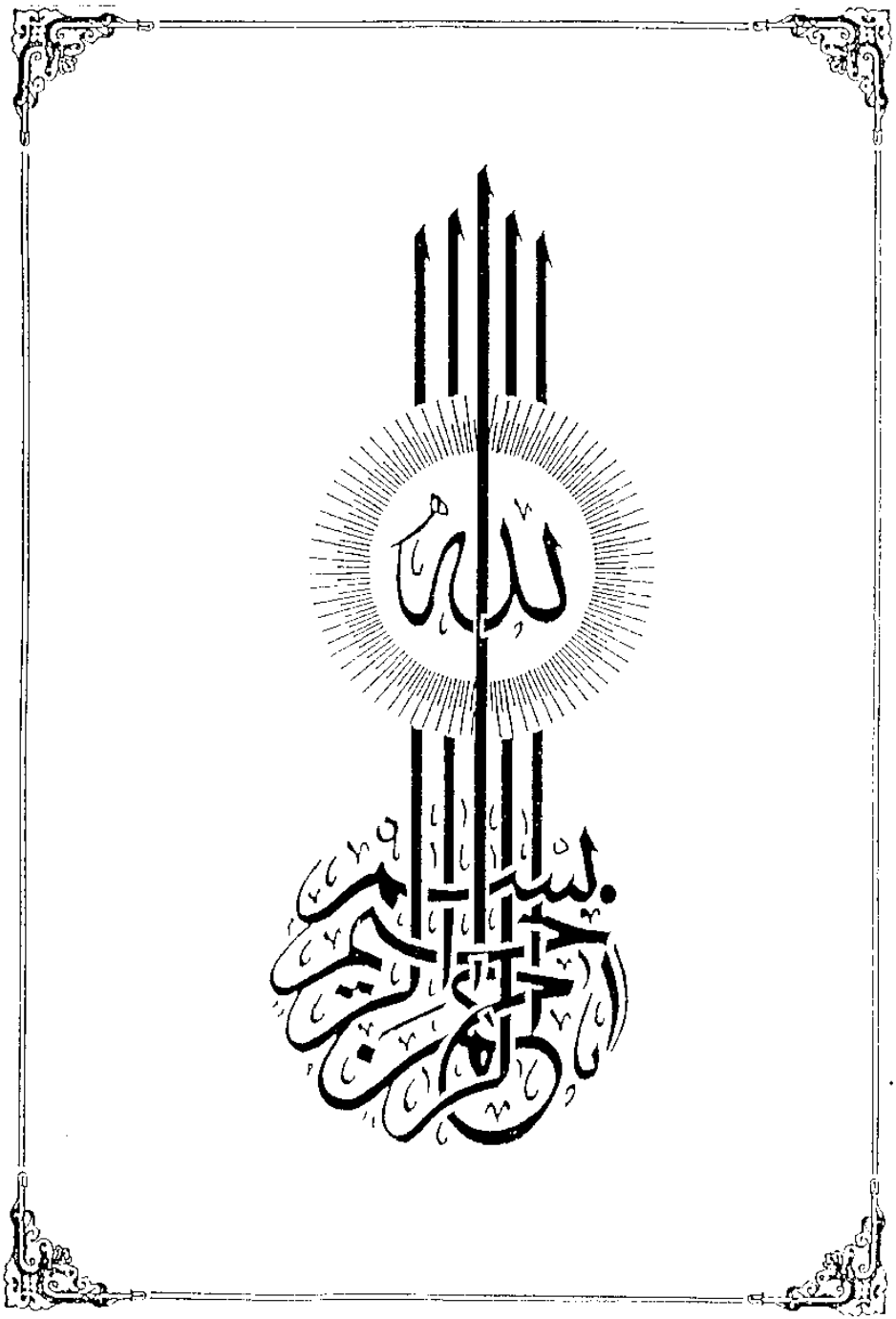

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AIM OF THE WORK

The aim of this work is to identify, characterize schistosome antigen(s) and express those antigens in appropriate vectors for the purpose of purifying sufficient amounts that may be used in animal protection studies and evaluation of vaccine potential. This will be facilitated by the use of recombinant DNA technology.

INTRODUCTION

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1.1. Schistosomiasis

Schistosomiasis is a chronic and debilitating parasitic disease affecting 200 million people throughout the world and is responsible for at least 500,000 deaths per year (Capron, 1992).

This disease is caused by members of adult worms of the genus *Schistosoma*, class:Trematoda, family: Schistosomatida. Five species of this sexually diversified trematode helminth can infect man but three principal ones are in the majority: *Schistosoma mansoni* in the South America and *Schistosoma japonicum* in the Far East. Whilst Africa and Middle East harbour both *Schistosoma haematobium* and *S. mansoni* in sometimes overlapping endemic areas (Bergquist, 1990).

Depending on the type of schistosomiasis, the clinical manifestations of the disease involve liver, intestinal and urinary complications resulting from reactions to schistosome eggs lodged in the tissues of infected people. In urinary schistosomiasis (due to *S.haematobium*) damage to the urinary tract is revealed by blood in urine. Urination becomes painful and there is progressive damage to the bladder, ureters and kidneys. Intestinal schistosomiasis (due to *S. mansoni* and *S.japonicum*) results in enlargement of the liver and spleen, damage to the intestine and hypertenstion of the abdominal and oesophageal veins. Bleeding from these vessels can be fatal (Bergquist, 1993).

The pathological reactions to schistosome infection which are generally related to the deposition of numerous eggs in the host tissues, mainly in the liver, cause severe granulomatous reaction (Arnon, 1991).

Egypt is one of the most highly endemic areas in the world (Abdel-Wahab, 1982). A WHO report in 1993, recorded that the total number of infected individuals in Egypt in 1990, was estimated to be 5-6 millions. Also reported that *S. mansoni* is more common than *S.haematobium* in the irrigated area of the Nile delta. In upper Egypt, *S.haematobium* infection is prevalent (WHO, 1993).

1.2. The life cycle of the schistosome parasite

People may become infected when they come in contact with water containing infective stages of the schistosome parasite. The larvae which are infective to humans are called cercariae. These are microscopic forms, measuring about 0.2 mm in length, which swim freely in water, and are able to seek out a person and penetrate the skin (skin cuts are not necessary for penetration). Cercariae are able to survive freely in water for approximately 24 to 72 hours, after which time, they die if they have not found a human or other suitable mammalian host to penetrate. Cercariae have long forked tails to assist them in swimming. When the cercariae enter a person's skin, they shed their tails.

After penetrating the skin, cercariae develop into another larval stage, the shistosomula, enter the venous circulation or the lymphatic vessels and pass through the right heart to the lungs and finally reach the liver, where they mature to adult worms. This maturation takes about 50 days for *S. mansoni* and about 80 days for *S. haematobium*. Each adult worm is either a male or a female (they are not hermaphrodites as are most trematode parasites). The longer and more slender female lies in the gynaecophoric canal of the broader and more muscular male. Adults of both sexes have oral and ventral suckers by which they attach to the inner walls of the blood vessels of the intestine or bladder. Adult worms may live up to 20 years or longer in the body.

After the worms mature, they mate in the liver and the worm pairs pass down the venous blood vessels from the liver to live in veins of the bladder (*S. haematobium*) or of the intestine *(S. mansoni*), where egg laying occurs. Aberrant localizations may also be found in various other parts of the body. Each female *S. haematobium* worm produces 150 eggs per day, while female *S. mansoni* produces 300 or more eggs per day. *S.haematobium* eggs have a terminal spine, while *S. mansoni* eggs have a lateral spine. The eggs pass through the walls of the bladder or intestine to enter the urine or feces.They pass from the body at the time of urination or defecation and hatch upon entering the water.The ciliated miracidia that hatch from the schistosome egg shells can live in the water for periods of 8 to 12 hours or more, after which they die if they have not found a proper snail host.

Schistosoma haematobium miracidia search out and specifically infect fresh water snails of the genus *Bulinus*, while *S.mansoni* miracidia specifically infect snails of the genus *Biomphalaria,* the miracidium penetrates the snail tissue, changes into a mother sporocyst or primary sporocyst near the penetration point (Burch and Bruce, 1990). The germ cells of the primary sporocyst increase in size, followed by multiplication of their number, then each cellular cluster, made up primarily of somatic and germinal cells, forms an individual secondary sporocyct that migrates towards the digestive gland of the snail. Once in the tissue of the hepatopancreas , the secondary sporocysts develop and increase greatly in size, simultaneously differentiation of the cercarial embryos occurs (the stage infective to man) (Webbe, 1965). The time involved for the miracidium to develop into the infective cercariae is approximately four weeks at 26-28°C. When mature, the cercariae emerge from the snail and enter the surrounding water to begin the cycle once again. One miracidium can produce several thousands cercariae, but each cercaria can produce

egg-induced granuloma formation may be possible (Capron *et al.*, 1990) The major advantage of a vaccine is that once immunity has developed, i could be long-lasting after one or two doses and would propably be given before the first, natural exposure to infection. It seems reasonable to expec that a single immunization would be sufficient to protect an individua living in an endemic area (Taylor, 1991). The key to develop an effectiv vaccine against schistosomiasis is dependent upon identifying an antige present on the surface of schistosomula or adult worms which can induc an antibody response that supports the eosinophil-dependent killing of th schistosomula (Cryz Jr, 1991).

1.4. Immunity in human schistosomiasis

The development of a vaccine is now a real possibility but, in orde to use such a vaccine most effectively, it is important to determine whic human immune responses should be elicited or avoided during vaccinatio that is, to understand the effector mechanisms of naturally acquired huma immunity (Butterworth, 1987).

Studies of age-specific prevalence and intensity curves o schistosome infection in endemic area, suggested the existence of naturally acquired immunity to superinfection in individuals bearing primary infection which, developed more rapidly and more effectively i communities exposed to high levels of infection (Butterworth and Hagan 1987). Epidemiological evidence indicates that during schistosomiasis, partial protection is attained, which is referred to as "concomitan immunity". This state describes the condition when an actively infecte host resists or partially resists, a subsequent challenge infection by the sam organism (Arnon, 1991).

Several studies have been also carried out for S. *mansoni* in Kenya and Brazil and for *S. haematobium* in Gambia (Hagan, *et al.*, 1985 and Lichtenberg , 1985). In each case, the conclusions have shown evidence for an age-dependent acquired resistance to reinfection that can be cleary distinguished from changes in exposure (Butterworth and Hagan, 1987).

Butterworth *et al.* (1985) examined the rate of reinfection of children following chemotherapy under conditions where the level of exposure was monitored. Two groups were identified. First, those presenting with high levels of reinfection and therefore identified as "susceptible" and second, those showing low levels of reinfection inspite of high exposure and therefore considered "resistant". The mean pretreatment intensities of the two groups were similar, but there was a marked difference in age indicating that the observed resistance was an age-dependent event and independent of levels of exposure.

1.4.1. Immune responses to schistosomes

In order to achieve protective immunity against schistosomiasis, one must induce resistance in the host towards the infective stage of the parasite, namely cercariae. Partial resistance against challenge infection has been achieved either by prior infection of mice with normal cercariae which led to development of a patent infection (concomitant infection) or by their exposure to irradiated cercariae (vaccine immunity) (McLaren and Smithers, 1987). In both cases, the immune response is directed against the infective schistosome larvae. This immunity is partially due to the skin-bound antibodies, as the skin is the first point of host parasite encounter during the invasive penetration (Arnon, 1991).

According to the experimental model used (rats or mice for instance) it is generally agreed that antibody-dependent mechanisms play a

major role in the expression of acquired resistance to schistosome infection. The major role of antibodies in protective immunity is to induce cytotoxic destruction of schistosomulum targets and although the significance of complement fixing antibodies is not yet resolved, antibody-dependent cell mediated cytotoxicity (ADCC) appears to be the main mechanism of killing parasites both in rat and human schistosomiasis (Capron *et al.*, 1980).

Extensive studies based on *in vitro* cytotoxicity assays and *in vivo* passive transfer experiments have revealed the existence of novel ADCC systems, involving inflammatory cells (macrophages, eosinophils, platelets) as cellular partners and IgE or anaphylactic subclasses of IgG as humoral components (Capron, 1992). As far as human studies are concerned most attention has focused on eosinophil and macrophages, while the role of neutrophils is controversial (Butterworth and Richardson., 1985).

In the case of eosinophils, killing can be mediated by both IgG and IgE isotypes although their relative effectiveness is dependent on the activation state of cells. Eosinophils from normal non-eosinophilic individuals mediate killing of schistosomula with IgG only, but cells recovered from eosinophilic individuals infected with helminth parasite, can kill schistosomula in the presence of IgE antibodies (Taylor, 1991).

So there is a strong association between helminth infections and increased total parasite-specific IgE levels and increased numbers of circulating eosinophils (Butterworth *et al.*, 1992). The preferential effect of eosinophils is associated with the capacity of these cells to degranulate upon contact with large nonphagocytosable, antibody-coated surfaces and to release onto such surfaces their granule contents, especially the toxic protein (Butterworth *et al.*, 1979 and McClaren *et al.*, 1981).

Activation of eosinophils is dependent upon production of cytokines with other functional properties, including tumor necrosis factor (TNF), interleukin-3 (IL-3) and interleukin-5 (IL-5) and also by other monocyte or lymphocyte derived mediators including eosinophil activating factor (EAF) and eosinophil cytotoxicity enhancing factor (E-CEF) (Butterworth *et al.*, 1992).

IgE antibodies are also responsible for mediating killing by resting macrophages and platelets although the mechanisms are different (Capron and Capron, 1987). In the case of macrophages, direct contact with the target is required, but platelets mediate killing through production of soluble toxins and direct contact is not required (Taylor, 1991).

Studies by Hagan and his colleagues (1991) on *S. haematobium* infections showed that IgE antibodies against adult worm and egg antigens progressively increase with age and are significantly correlated with a lack of subsequent reinfection. These evidences support the idea that IgE plays a protective role in mediating immunity to helminth infection in humans.

In addition, there is association between lack of reinfection and IgA responses against adult worm antigens. These IgA responses increase particularly during adult life (from the age of 25 years onword) rather than late childhood as in the case of IgE responses (Butterworth *et al.*, 1992).

All children, including the younger ones who remain susceptible to reinfection after treatment, show a wide range of immune responses to antigens from various parasite stages. Studies on antibody responses in such children yielded initially no correlates of immunity. Instead, some antibody responses were found to correlate strongly with susceptibility to reinfection (Butterworth *et al.*, 1988). These included antibodies of the

IgM and IgG2 isotypes that recognized carbohydrate epitopes expressed both in eggs and on the surface of the developing schistosomula. These antibodies cross-react with carbohydrate epitopes on polysaccharides or glycoproteins at the schistosomulum surfaces, and not only fail to mediate ADCC reactions by eosinophils or other cells but may indeed block the binding of antibody isotypes, separately elicited against the same or closely adjacent epitopes. These blocking antibodies then decline with age, permitting the binding and action of effector antibodies and hence the expression of immunity (Butterworth *et al.*, 1992).

Khalife *et al.* (1986) demonstrated directly that purified IgM would block the eosinophil-dependent killing of schistosomula mediated by IgG antibodies from the same sera. Subsequently, following purification of individual IgG subclasses, they found that IgG1 and IgG3 would mediate eosinophil killing of schistosomula. IgG4 consistently blocked killing while IgG2 would either mediate or block killing, depending on the state of activation of the eosinophils.

The capacity of an IgG2a rat monoclonal antibody to mediate eosinophil killing of schistosomula *in vitro*, can be blocked by an IgG2c monoclonal antibody which shares specificity for the same 38,000 M_r glycoprotein antigen (Grzych *et al.*, 1984).

1. 5. Immune evasion by the schistosomes

Infectious parasites have evolved a diverse array of specific and general adaptations to evade the host immune system. The defence strategies used by parasite, constrained by replication rate and extracellular locale, can be divided into three classes: avoiding initial induction of damaging immune responses; compromising selected arms of the immune