EFFECTIVE DIAGNOSIS OF *SCHISTOSOMIASIS HAEMATOBIIUM* BY IMMUNOMAGNETIC BEAD ELISA TECHNIQUE USING SUPER-PARAMAGNETIC NANOPARTICLES

A Thesis

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By

*Amany Ahmed Saied Ahmed Emam*

B.Sc. Cairo University

Department of Zoology
Faculty of Science
Cairo University

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بكالوريوس علم

قسم علم الحيوان
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Human schistosomiasis is a chronic, debilitating parasitic disease and is caused mainly by three species of the genus *Schistosoma* (S.): *S. haematobium*, *S. japonicum* and *S. mansoni* (He et al., 2005). More than 600 million people are at risk with about 200 million actually infected in 74 countries mainly in the tropics and subtropics (Ruelas et al., 2006). *Schistosomiasis haematobium* is an important public health problem in Africa and the Middle East affecting more than 110 million people in rural, agricultural and peri-urban areas (WHO, 2008). Schistosomiasis is second only to malaria in terms of public health importance (Abdulla et al., 2007). It is associated with a variety of clinical syndromes that may lead to severe morbidity (Bahgat et al., 2010). The ancient Egyptians contracted the disease more than 4000 years ago (El-Zayadi, 2004).

Adult worm pairs of *S. haematobium* are found in copula within venous plexuses surrounding the bladder and ureters. Hundreds of eggs are laid by each female worm per day, and these gradually find their way into the lumen of the bladder (Blanchard, 2004). The disease is characterized by painful micturition, dysuria, hematuria, proteinuria and the presence of schistosome eggs in the urine of infected persons (Bosompema et al., 1996; Conor et al., 2002). In the later stages immune-mediated granulomatous response to parasite eggs lead to granuloma formation in the lower urinary tract; which is the main cause of the pathology in the bladder (Pearce et al., 2002). Schistosomiasis is associated with debilitating morbidity manifested by sequelae such as iron deficiency anemia, cognitive impairment, lassitude, growth stunting (Savioli et al., 2004) and predisposition to cancer of the bladder especially in adults (Michaud, 2007).

Routine diagnosis of *Schistosoma haematobium* infections can be done by detection of eggs in urine samples where eggs can be demonstrated because the volume of urine usually screened is relatively large. In addition, urine does not contain fibre-like structures as in stool which may hamper the recognition of eggs (Van Leishout et al., 2000). However, the number of eggs counted in urine is strongly influenced by the protocol of sample collection, as most eggs are found around noon after physical exercise in combination with fluid intake, and in the last drops of micturition (Savioli et al., 2004). Besides, routine microscopic technique is not sensitive enough, as it is difficult to find eggs in the urine of people with a low worm load, those with infections less than one month duration or in patients with
chronic infections, where egg production and excretion is low (Lengler et al., 1991; Muller, 2002).

Schistosoma antibody detection assays, though very sensitive particularly in individuals from endemic areas, do not however differentiate between active and past infection and do not correlate with intensity of infection (Van Leishout et al., 2000). In addition, elevated antibody levels are still detectable many years after treatment (Ross et al., 2001).

Antigen detection could be used in routine screening for case detection in low transmission areas or detection of residual infections in very low transmission areas in order to eliminate the parasite reservoir and aid interruption of transmission. In *S. haematobium* infection, antigen levels were found to be significantly correlated with the egg excretion and decreased rapidly following successful treatment (Van Lieshout et al., 1994; WHO, 2000). Detection of schistosomal antigens in serum and urine is a powerful immunodiagnostic tool and is considered as an alternative to egg counts in faeces (De Jonge et al., 1988; 1990; Van Lieshout et al., 1992), and urine (Kremsner et al., 1994). As the sensitivity of both antigen detection and egg counts is limited when the intensity of infection is low (De Jonge et al., 1991), a diagnostic technique with increased sensitivity is desirable. A variety of antigens are secreted and excreted by parasites present in the blood, faeces, urine and other fluids of the infected host. These antigens have potential for use in immunodiagnosis and vaccine development (Abdel-Rahman, 1999).

Evaluation of efficacy of excretory/secretory (E/S) antigen by enzyme linked immunosorbent assay (ELISA) (Osman et al., 1995) revealed that, the crude preparation had 100% sensitivity, 94% specificity and 98% accuracy at cut off level of 0.3 in acute cases and positive results in 77% of chronic cases.

In this study, rabbit polyclonal antibodies (pAb) was used against E/S antigen of *S. haematobium* as a capture antibody and anti-E/S rabbit polyclonal antibody labeled horseradish peroxidase (HRP) as a detecting antibody. This study was conducted on 100 *S. haematobium* infected patients from endemic areas in El fayoum Governorate and from out clinic patients and hospital at TBRI and El-Azhar University Hospital. Patients were diagnosed by ERCP or finding characteristic eggs in urine samples collected and 63 patients were infected with other parasites.
(Fasciola, hookworm, hydatid and trichostrongyloides). In addition, 35 individuals of the medical staff at TBRI served as parasite free-healthy negative control.

The magnetic bead immunoassay combines the use of magnetic beads with a high binding capacity as a solid phase and the rapid reaction kinetics of solutions with the simple separation of bound and unbound materials on the solid phase, which provides the chance of enhancing the sensitivity of antigen detection (Gundersen et al., 1992; Ndhlovu et al., 1995).

The immunomagnetic bead ELISA (IMB-ELISA) is found to provide higher specificity and sensitivity compared to a microplate-based ELISA technique.

**Aim of work**

Evaluation the role of prepared E/S S. haematobium antigen in the detection of the infection through raising polyclonal anti-Schistosoma antibodies. Comparative evaluation of S. haematobium E/S antigen with sandwich ELISA in relation to immunomagnetic bead ELISA technique.
1.1. Schistosomiasis

Schistosomiasis was discovered by Theodore Bilharz, a German surgeon working in Cairo, who first identified the etiological agent *Schistosoma hematobium* in 1851 (Nour, 2010).

Schistosomiasis is a parasitic disease caused by blood flukes (trematodes) of the genus *Schistosoma* (*S*.). An estimated 700 million people are at risk of infection in 76 countries, considered endemic, as their agricultural work, domestic chores, and recreational activities expose them to infested water (WHO, 2008). More than 207 million people are infected with schistosomiasis; 85% live in Africa (WHO, 2008). After malaria, and intestinal helminthiasis, schistosomiasis is the third most devastating tropical disease in the world (WHO, 2008). Sometimes referred to as bilharzias, bilharziasis, or snail fever.

*S. haematobium*: the vesical blood fluke, a species with terminally spined eggs that occurs as a parasite in the portal system and mesenteric veins of the bladder (causing human *schistosomiasis haematobium*) and rectum; common in the Nile delta but is found along waterways, irrigation ditches, or streams throughout Africa and in parts of the Middle East. The intermediate host is *Bulinus truncatus* in Egypt; elsewhere, other snails of the subfamily Bulininae (*Bulinus, Physopsis, Pyrgophysa*) are involved.

1.2. Epidemiology and prevalence of *S. haematobium* in Egypt

Schistosomiasis is one of the world’s major health problems. Blanchard (2004) stated that 85% of schistosomiasis (*mansoni* and *haematobium*) is found in sub-Saharan Africa, with additional foci in Egypt along the Nile, and in Yemen. It was estimated that more than 200,000 deaths per year are due to schistosomiasis in sub-Saharan Africa (WHO, 2005).

Schistosomiasis transmission depends, unlike the transmission of malaria and other insect-transmitted diseases, on the active role of the human host in the transmission process, through excretory contamination of snail habitats and direct contact with infective water (Kloos and Thompson, 1979). This ecological relationship thus makes schistosomiasis a disease closely linked to rural water
resources development, population increase, inadequate sanitation and lack of effective medical treatment (Kloos and David, 2000).

Geographic distribution of *S. haematobium* includes large parts of northern, central and southern Africa, Nile valley in Egypt and Sudan as well as sections of Saudi Arabia, Madagascar, Mauritius, Syria, Turkey, Iraq and Iran (Mehlhorn, 2008). The Nile River has been an epicenter for schistosomiasis since antiquity. In 1980, an estimated 10% of the 200 million persons infected with *Schistosoma* were Egyptians (Abdel-Wahab et al., 1980).

During his extensive country-wide survey for schistosomiasis 70 years ago, John Scott showed that *S. haematobium* was the only species transmitted in Middle and Upper Egypt, south of Cairo, and both it and *S. mansoni* were endemic in the Nile Delta (Scott, 1937). With the completion of the Aswan High Dam in the 1960s, the flow of the Nile was controlled and summer floods no longer occurred. This provided a selective advantage to the intermediate host for *S. mansoni*, *Biomphalaria alexandrina*, over the host for *S. haematobium*, *Bulinus truncates*. By 1979, there was an inversion from Scott’s survey 50 years earlier in prevalence of *S. mansoni* and *S. haematobium* (from 3% and 73% to 74% and 2%, respectively) (Abdel-Wahab et al., 1979). This reversal in human infection rates apparently followed a similar change in abundance of snail vectors for the two parasites. This change was believed to be caused by less silt and variability in velocity and volume of water (El-Khoby et al., 2000a).

In the mid 1990s, 12% of the Egyptian population was infected with *S. mansoni* and 6% with *S. haematobium*. But in smaller agricultural villages, where life continues much like in ancient Egypt, prevalence rates over 50 percent are still found (El-Khoby et al., 1998).

The following table (1) shows records of the Ministry of Health and population (MOHP) illustrating the average prevalence of *S. haematobium* in Egypt since 1935 to 2008. It shows that the prevalence of *S. haematobium* in Egypt has declined from 48% in 1935 to 0.6% in 2008.
Table (1): Overall national prevalence of *S. haematobium* in Egypt (MOHP records, 2009).

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<td>Prevalence</td>
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<td>Prevalence</td>
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Human waste in water containing intermediate hosts is the most important epidemiological factor in schistosomiasis, together with the availability of suitable snail species which determine endemicity of a particular species of *Schistosoma* (Grassi et al., 2001).

Rates of infection depend on the type of irrigation used, with the highest rates in localities where perennial irrigation prevailed and the lowest rates in basin irrigated areas. Rates were also higher in smaller villages, where nearly all residents are farmers poverty is most severe, and contact with the canals is most intense (El-Khoby et al., 2000b).

The role of reservoir hosts and strains of parasites has some importance as epidemiological factor, depending on the species. *S. haematobium* is more host specific than *S. mansoni*, and is thought that no natural reservoir hosts exist for it (Chilton et al., 1999).

**1.2. Susceptibility factors for infection**

Clearly, economic and education level of a population will influence transmission of the disease, and age and sex are important factors as well (Hagan and Wilkins, 1993).

In areas where schistosomiasis is endemic, there is an obvious pattern of age-dependent intensity of infection; individuals who are below the age of puberty carry the most parasites, and those in older age are generally less heavily infected (Butterworth et al., 1994; Pearce and McDonald, 2002).

It should be noticed that not only resistance to infection is age-dependent, but reinfection is also related to age, with children being more susceptible than adults (Zhang and Mutapi, 2006).
Males usually show the highest rates of infection, the most intense infections and the most hazardous age is the second decade of life (Hagan and Wilkins, 1993).

Some specific occupations are strongly associated with schistosome infections in endemic regions. These include farming, fishing (fresh water) and working in irrigation canals. Performing laundry or other domestic activities such as washing clothes, washing cooking materials or bathing also contribute to increased rates of infection (Steinmann et al., 2006).

A comparison of immune responses between individuals who are susceptible and those who are resistant to infection has shown that there is a correlation between immunoglobulin-E (IgE) responses to worm (not egg) antigens and immunity, which implicates IgE in the protective effector mechanism (Dunne et al., 1992; Demeure et al., 1993).

Mutapi et al. (1997) indicated that the production of anti-soluble egg antigen (SEA) IgE in the high infection area was significantly higher than in the low infection area, whereas the level of anti-worm IgE was lower in the high infection area than in the low infection area.

It has also been proposed that acquired immunity develops as a function of cumulative exposure (duration and frequency) to parasite antigens (Woolhouse and Hagan, 1999). Acquired immunity develops quicker in exposed individuals in areas of high-transmission than in those in areas of lower transmission because the population is exposed to a higher level and a greater diversity of antigens in a high transmission area (Mutapi et al., 1997; Woolhouse and Hagan, 1999).

In addition, experimental work done in hamsters has indicated that animals with concomitant Leishmania and Schistosoma infections produce significantly higher level of IgA, IgG and IgE antibodies against each parasite compared with animals infected with just one parasite species (Mangmoud et al., 1997). Mutapi et al. (2000) stated that children infected with schistosomes and malaria parasites produced significantly higher concentrations of IgE and IgG3 against schistosome egg antigen compared with children infected with schistosomes only.

Host genetics can also influence the differentiation of parasite-specific T helper 2 (Th2) cells, thus influencing the immune response to the parasite (Marquet et al., 1996). Studies in Brazilians and Kenyans infected with Schistosoma
have suggested that there is some genetic restriction with regards to the ability of the host to recognize different epitopes, to mount a specific immune response and to produce an isotype profile against the same antigen (Mutapi, 2001).

1.3. Life cycle

The life cycle of all schistosomes follow the same pattern and is characterized by alternation of generations with sexual non-multiplying reproduction in vertebrate host and asexual-multiplying reproduction in the intermediate host. Mature eggs excreted from the definitive host hatch only in fresh water, releasing a ciliated meracidium. This first free living larval stage remains infective for up to 48 hours (hr) and must find an appropriate intermediate host (snail) within this period. For life cycle to continue, the miracidium penetrates the soft parts of the compatible fresh water snail, *Biomphalaria alexandrina* in *S. mansoni*, *Bulinus truncatus* in *S. haematobium* and *Onchomelania* species in *S. japonicum*, where it transforms into a primary sporocyst near the site of penetration by asexual multiplication that in turn develops into daughter sporocysts. The daughter sporocysts migrate to the snail’s digestive glands where they grow and multiply in about 8-10 days. After that, they release many unisexual free swimming cercariae, “the infective stage” through the snail tissue into the water in a light-dependent process. When a definitive host is in contact with water contaminated with cercariae, they penetrate the unbroken skin within few seconds; they stimulated to do so by the secretion of certain fatty acids and their derivatives by the mammalian skin (Stirewalt et al., 1983).

On penetrating the host skin, the cercariae lose its tail and transforms from trilaminate form to heptalaminate form (schistosomulum) within 3-6 hr in the stratum corneum (Nanduri et al., 1991). Migration of schistosomula from the skin to the hepatic portal system is entirely intravascular taking between 8 and 20 days to complete (Miller and Wilson, 1980). The schistosomula enter the initial epidermal layer of the skin very rapidly (in less than 30 minutes (min)). They remain within the epidermis from 48-72 hr before entering the basement membrane and the dermis where they remain for about 18 hr to begin the migration phase, about 10 hr to locate the venules and further 8 hr to penetrate the venules wall (Wilson, 1987; Curwen and Wilson, 2003). Schistosomula enter in the blood system directly, or indirectly
via lymphatics (enter the circulation via the thoracic duct) (Wheater and Willson, 1979; He et al., 2002). They are transported passively by the blood flow via the left side of the heart and the lungs. They pass through right heart to the lung and begin to develop in a period of 3-4 days to adapt for inward migration. Schistosomula passing to the venous compartment of the lung and carried to the left heart and dispersed to systemic organs, where they traverse the capillaries and reach the venous compartment, 30-35 hr, to be carried back to the lung (Wilson and Coulson, 1986). Parasites arrive to splanchnic vasculature of the hepatic portal system, eventually reaching the sinuses of the liver where worms remain for a period of 3 weeks (wk) of development then transform into adult worms (Combes, 2002). Young adult pairs after 4-5 wk post infection (PI) for S. mansoni and S. japonicum, 1 or 2 wk later for S. haematobium, then the male which is flattened clasps the long cylindrical female in the gynaecophoric canal. In single sex infection, the sexual organs of female worms are underdeveloped leading to the hypothesis that one or more male factors are essential for complete maturation of the female (Hernandez et al., 2004). After pairing the couple migrate against the blood flow of hepatic portal system to oviposition site according to species of schistosomes, either to the mesenteric veins (inferior mesenteric venous plexus in the region of rectum and pelvic colon) in S. mansoni (superior and inferior mesenteric venous plexus) in S. japonicum and S. intercalatum or vesicle and pelvic plexus (around bladder) in S. haematobium infection. Then females begin to lay eggs in these vessels (liver, intestine, and bladder) and the life cycle continues. The female lies from 200 to 2000 eggs per day over of average of 5 years, according to species (WHO, 2002). Many eggs are trapped in the tissues and are the cause of the observed pathology. Whereas, about half of the eggs penetrate through the bladder or intestinal wall and are excreted or to the external environment via the urine or faeces to continue the life cycle (Kahama et al., 1998). The length of period between penetration of cercariae and the first passage of eggs in excreta (prepatent period) is uniform, but there is some variation between species and among strains within species (Mahmoud, 2001).
1.4. Schistosomiasis pathology

Approximately 200 million persons are infected with schistosomes. Of those infected, a small proportion develop serious chronic disease, usually after years of intensive exposure and infection. *S. mansoni* and *S. japonicum* reside in the mesenteric veins and produce liver fibrosis, which results in portal hypertension and bleeding esophageal varices but little hepatocellular dysfunction. *S. haematobium* resides primarily in the pelvic veins and produces mass lesions in the bladder and ureters, which lead to hydroureter and hydronephrosis. The intensity of infection is a major factor determining development of disease, but differences in worm strain and host response may also be important. In acute schistosomiasis there is an intense response to the parasite, which is suppressed as the infection becomes chronic. The marked inflammatory response seen in early and acute schistosomiasis becomes less intense and fibrotic lesions predominate. The recent advent of safe, effective, and easily administered chemotherapeutic reagents will aid in the control of schistosomiasis (Nash et al., 1998).

The different *Schistosoma* spp. affect their final host by a variety of life-threatening diseases in a relatively specific manner. Examples of these diseases are genitourinary diseases which are caused by *S. haematobium*, gastro-intestinal and hepatic diseases via the infection with *S. mansoni* and *S. japonicum*. Neuroschistosomiasis which develop when the schistosome egg find its way to the brain in case of *S. japonicum* and spinal cord for *S. mansoni* and *S. haematobium* (Olson et al., 2002; Ross et al., 2002; Christophr, 2005; Ferrari, 2004; 2008).

1.4.1. Acute schistosomiasis or Katayama syndrome

Tissue migration of schistosomal larvae may cause a hypersensitivity reaction. Any species of *Schistosoma* can cause it. Although most clinical manifestations are benign, some are severe and may require hospitalization. If acute schistosomiasis (AS) is not suspected clinically and treated appropriately, it can result in severe morbidity or death. Nonimmune travelers are especially prone to this disease manifestation. After a single exposure to a freshwater pond in Tanzania, 86% of tourists developed as symptoms including cough, fever, and fatigue (Leshem et al., 2008). Symptoms usually appear 2-12 wk after exposure.
1.4.2. Chronic schistosomiasis

Most patients are asymptomatic or mildly symptomatic and do not require medical attention. Only a small proportion of the endemic population harbors a heavy worm burden that later leads to clinical complications.

1.4.3. Gastrointestinal schistosomiasis

The most common complication is periportal fibrosis, also termed Symmers clay pipestem fibrosis. This leads to portal hypertension and gastrointestinal hemorrhage. Liver failure is uncommon, except in persons with concomitant chronic hepatitis or cirrhosis. Of those with *S. mansoni*, *S. japonicum*, and possibly *S. mekongi*, 4-8% develop hepatosplenic disease (Lapa et al., 2009).

People co-infected with either hepatitis B or C and *S. mansoni* have been shown to have rapid progression of liver disease.

1.4.4. Urinary tract schistosomiasis

This can lead to renal failure due to obstructive uropathy, pyelonephritis, or bladder carcinoma (occurring usually 10-20 years after the initial infection). In addition, immune complexes that contain worm antigens may deposit in the glomeruli, leading to glomerulonephritis and amyloidosis.

1.4.5. Female genital schistosomiasis

*S. haematobium* causes lesions in the female lower genital tract (ie, cervix, valva, vagina). Female genital schistosomiasis (FGS) has been identified as a major social and medical problem that may facilitate the spread of some sexually transmitted diseases such as Human immunodeficiency virus (HIV) and human papillomavirus (HPV) (Mosunjac et al., 2003).

1.4.6. Coexistence of sexually transmitted infection and urogenital schistosomiasis

One study found that, in women with *S. haematobium* infection in Madagascar, 35% may have co-existing sexually transmitted infections like *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Mycoplasma genitalium*, or *Trichomonas vaginalis*, as compared with 17% of the men. This is found to be more common in
younger populations (15-24 years) than older populations. The association became stronger with greater parasite burden (Leutscher et al., 2008).

1.4.7. Pulmonary arterial hypertension in Schistosomiasis

This is an important complication that develops in about 7.7% of patients with hepatosplenic disease in S. mansoni, S. japonicum and possibly S. mekongi infections. The prevalence of the disease worldwide is estimated to exceed 270,000 individuals (Lapa et al., 2009).

1.4.8 Central nervous system (CNS) schistosomiasis

Most cases of cerebral schistosomiasis are observed with S. japonicum. CNS involvement occurs in 2-4% of all S. japonicum infections. One million people in China are estimated to be infected with S. japonicum. Cerebellar nodular enhancing lesions can occur as well with S. japonicum (Wan et al., 2009). S. japonicum causes 60% of all Schistosoma brain infections because of its smaller egg size (Walker and Zunt., 2004).

However, CNS schistosomiasis can also occur with other species. Spinal schistosomiasis usually presents as transverse myelitis and is primarily due to S. mansoni infection because of the larger egg size. S. haematobium can infect the brain or the spinal cord (Walker and Zunt, 2004). The distribution of S. mekongi is limited to the Mekong River basin in Laos and Cambodia, where some 140,000 people are estimated to be at risk for this infection. Temporal mass causing paraesthesias of the arm and leg with dysphasia has been described with S. mekongi infection (Houston et al., 2004). Neurologic symptoms can develop months after the infection. Cauda equina syndrome, anterior spinal artery syndrome, and quadriaparesis can occur. Most of the lower spinal cord is affected (Walker and Zunt, 2005).

1.4.9. Schistosomiasis in pregnancy

This has been associated with anemia and low birth weight (Friedman et al., 2007).
Immune response against schistosomiasis

The process involved in immune response against *Schistosoma* is complex. Not only due to the antigen variation during the life cycle and the intensity of expression of antigenic component in the parasitic organism, but also due to the mechanism by which *Schistosoma* evades host immune system (*Zhang and Mutapi, 2006*).

Three main findings piqued the interest of immunologists in schistosomiasis: the immune response is intimately involved in the development of many of the pathological changes that accompany infection; infected individuals can have resistance to superinfection; and schistosomes survive for years in the host despite a strong immune response (*Pearce and MacDonald, 2002*).

Humoral immune response

Polyclonal B-cell activation was found to be responsible for hypergamma-globulinaemia in urinary schistosomiasis and could have arisen from the direct effect of *S. haematobium* on B-lymphocytes. Similarly, elevated levels of IgG1, IgA and IgE in urinary schistosomiasis are associated with Th2 responses (*Arinola and Salimonu, 2004*).

Cox (1996) reported that in *S. haematobium* infections, the protective antibody is IgE and the blocking antibody is IgG4. Levels of the different classes of immunoglobulins vary at the subsequent stages of urinary schistosomiasis. Th2 cells producing interleukin (IL) IL-4 and IL-5 seem to play critical roles in developing high level of anti-parasite IgE in schistosomiasis patients. Antibodies of IgE isotype is involved in the protection against schistosomiasis by mediating macrophage toxicity (*Dutra et al., 2002*).

IgE as the major antibody in Th2 response is controlled by macrophages, dendritic cells and T cells. Its role in allergic inflammation and hypersensitivity is achieved through activating mast cells and basophils which release histamine, tumour necrosis factor (TNF), IL-4, IL-13 and IL-5. IL-5 promotes the proliferation and differentiation of eosinophils (*Zhang and Mutapi, 2006*).
The observed parallel increase in the production of eosinophils and IgE against *Schistosoma* promoted researchers to investigate the interaction between eosinophils and IgE antibodies. Although in most cases, IgE produced by B cells stimulates mast cells and basophils to release mediators which stimulate eosinophil differentiation and induce eosinophil cytotoxicity, IgE can directly affect eosinophils by binding to receptors on eosinophils. These receptors can mediate schistosomula-specific eosinophil-dependent cytotoxicity by signaling the release of eosinophil peroxidase (EPO) (Gounni et al., 1994; Zhang and Mutapi, 2006).

The characteristic pattern of *schistosoma* infection-age curve is related to the effector mechanism of protective IgE antibodies and class switch between IgE/IgG4. Children produce significant amounts of IgG4 which is thought to interfere with complement activation by IgG1 and block mast-cell degranulation by competing with specific anti-parasitic IgE for antigenic worm antigen (Hagan et al., 1991). Therefore, the slow achievement of epidemiologically significant immunity may reflect a delayed development of the protective IgE response and early production of IgG4 which blocks the activity of anti-parasitic IgE (Butterworth et al., 1996).

Other antibodies, including IgM and IgG2, which are elicited against egg polysaccharide antigens and carbohydrate epitopes expressed at or released from the surface of the young schistosomulum, also appear to block the expression of protective antibodies (Butterworth et al., 1996).

Serum IgA and IgM were found to be elevated in the acute stage of urinary schistosomiasis (Hagan and Abath, 1992), therefore suggesting the diagnostic use of these immunoglobulin classes. Some researchers suggest a role of *Schistosoma*-specific IgA in mediating protective immunity in people, or in the slow release of somatic antigens of dying worms (Woolhouse and Hagan, 1999; Capron et al., 2005).

A study has implicated the involvement of *S. haematobium* parasite in the production of rheumatoid factor (Arinola and Salimonu, 2000). Nevertheless, an aetiological role of these autoantibodies in pathology (e.g. glomerulonephritis, anaemia) has not been directly established (Arinola, 2005).
Complement factors and immune complexes

Complement proteins in the plasma act through a cascade of reactions to attack extracellular pathogens. The terminal components in the cascade are designed to create pores in the plasma membrane of the target organism to debilitate and kill. The effector functions of complement can be activated through three pathways. The classical pathway is triggered when antibody binds to antigen. The other two pathways are activated by an interaction with pathogen surfaces: the mannose-binding lectin (MBL) pathway is activated by the binding of MBL to carbohydrates on the pathogen surface; the alternative pathway is initiated when a spontaneously activated complement component binds to the pathogen surface (Skelly, 2004).

In theory, schistosomes could activate all three of the pathways. Tarleton and Kemp (1981) documented the presence of Ig on the tegument of S. mansoni, and this could from a focus for complement (C) C1q binding and subsequent complement attack through the classical pathway. Complement component C3 is pivotal in all three pathways of complement activation. Using specific anti-mouse C3 antisera, C3 was shown to be associated with the adult female tegument (Kabil, 1976) and male tegument (Rasmussen and Kemp, 1987).

Schistosoma contains proteins that bind in vitro to the complement proteins C1, C2, C8 and C9 and may thus block the complement cascade at multiple steps (Laclette et al., 1992; Inal and Sim, 2000).

The components of schistosomule glycocalux have the ability to activate complement pathways to generate chemoattractants that promote adherence of phagocytic cells. Despite the activation of complement system on the surface of schistosomule, it is not damaged or killed (Warren, 1982).

Immune complexes due to Schistosoma antigens have been related to the amount of complement components or products in the serum of the host. It has been known that adult Schistosoma releases antigenic materials into the circulation of mammalian hosts. Free Schistosoma antigens are present only in massive infections but in the more moderate infection, which is normally found in nature, circulating antigens immediately combine with antibody forming complexes (Arinola, 2001).
Cell-mediated pathology

Only the egg antigens are important in the pathogenesis of granuloma disease (Smith et al., 2005). Most of the granulomatous pathology to schistosome eggs is due to hypersensitivity reactions. These focal reactions represent the host’s attempt to wall-off, contain, and perhaps destroy schistosome ova deposited in tissues (e.g. liver, intestine and bladder). Nevertheless, as these lesions stimulate extensive tissue fibrosis, they are at the same time harmful to the host and are the primary cause of schistosomal disease (Arinola and Salimonu, 2003).

In human schistosomiasis, Th1 cells produce high level of interferon gamma (IFNγ) and IL-2 at the acute stage of infection but production subsequently diminishes at the chronic stage, when IL-4 and IL-5 are predominant (Pearce et al., 1991). A study in Nigeria showed that immune responses controlled by Th2 cytokines predominate during urinary schistosomiasis though Th1-mediated immune responses are present (Arinola and Salimonu, 2003).

The cluster of differentiation (CD) CD4 T-cell response that is induced by egg antigens orchestrates the development of granulomatous lesions which are composed of collagen fibres and cells, including macrophages, eosinophils and CD4 T cells around the individual eggs (Dunne and Pearce, 1999 ; Dombrowicz and Capron, 2001).

The immunology of granuloma formation is complex and involves the activity of both major subsets of CD4$^+$ T helper cells. SEA is a powerful inducer of Th2 responses, but Th1 also plays a role. The cellular response is initiated and controlled by the cytokines released (Arinola and Salimonu, 2003).

Mechanism of schistosomular killing by eosinophils

Complement components in the presence of antibodies are activated on the surface of schistosomula to attract eosinophils, which adhere via C3 receptor interaction (Smithers and Doenhoff, 1982).

Following adherence, eosinophils begin to flatten against the parasite surface to make intimate contact with the tegumental surface. The eosinophil granules, that contain hydrolytic enzymes in addition to major basic protein, move towards the basal region of the cell fuse together to form vacuoles connecting with basal plasma
membrane and release vacuolar contents onto the surface of the parasite (Kazura et al., 1997).

The granules contain a variety of mediators such as a major basic protein, eosinophil cationic protein, eosinophil derived neurotoxin, eosinophil protein x and EPO. Freeze fracture studies have shown that the outer bilayer of the double outer membrane is damaged first and becomes locally separated from the inner bilayer that is attacked by eosinophil secretions. Tegumental vacuolation follows and as a result of permeability changes in the remaining membrane, small lesions are formed through which eosinophil migrates. After this, eosinophils flatten as they move between the tegument and the underlying muscle layers. In this way, eosinophils strip the tegument away from the body of the worm (Jonge et al., 1984; Ackerman et al., 1985).

**Role of neutrophils, macrophages, mast cells and platelets in immunology os schistosomiasis**

In a study done by Arinola and Salimonu (1999) they showed that neutrophils are responsible for less damage of the schistosomule when compared with the damage caused by eosinophils. Though a neutrophil possesses more fragment of crystallization (Fc) receptors than an eosinophil, the antibody mediated adherence of neutrophil to schistosomular surface is not permanent and does not result in significant damage and killing.

Neutrophil damages schistosomula by both direct physical means using pseudopodia, and enzyme action of the vacuoles. The neutrophil plasma membrane fuses with the inner membrane of the parasite. Following this adherence, the schistosomular tegument exhibit isolated regions of disintegration and adherent neutrophils start to push pseudopodial processes into the adjacent regions of the remaining tegumental cytoplasm. These pseudopodia do not penetrate beneath the basal membrane of the tegument. Neutrophils appear to rely on osmotic and enzyme-induced local damage (Caulfield et al., 1980; Arinola and Salimonu, 1999).
Macrophage-mediated killing appears to operate through two distinct mechanisms. One of which is specific and involves anti-parasite IgE antibodies especially in the form of immune complexes (Capron et al., 1987; Dessaint and Capron, 1989). The other mechanism is non-specific and antibody independent and is mediated by macrophages that have been activated by IFN-γ released from Th1 cells. The activated macrophages produce toxic molecules including reactive oxygen intermediates, superoxide, hydrogen peroxide, nitric oxide and tumour necrosis factor which destroy the target cells (Cox and Wakelin, 1998).

In 1995, Rashed et al. observed mast cells and eosinophils in schistosome egg granulomas in the liver of mice and hamsters. The platelets are also effector cells in antibody dependent cellular cytotoxicity reactions against parasites. The killing functions of these cells against schistosome larvae are however, dependent on IgE antibodies and Fc receptors belonging to the same class (Capron et al., 1987).

**Mechanism of immune evasion by Schistosoma**

Jenkins et al. (2005) demonstrated the ability of schistosomes to down regulate the host’s immune response in order to promote their own survival. This down regulation is also important for the host, as it limits the extent of pathology.

For survival in the complex immunological environment of vascular system of the definitive host, schistosomal parasites have evolved numerous strategies to confront host defence mechanisms, including antigenic mimicry, membrane turnover, essential function of immunomodulatory molecules and proteases, unique biophysical properties of the tegument and modulation of expression of surface antigens (Abath and Werkhauser, 1996).

Modulation usually occurs during the chronic phase of infection; however, it can also occur much earlier at the time of cercarial penetration of the skin. The parasites secrete modulators that act as the physiological actors at the interface between the parasite and the host (Johnston et al., 2008).

The immunomodulatory actions of invading cercariae are mediated by the molecules they release as they penetrate the host skin (e.g. the glycocalyx and acetabular secretions). The ultimate goal of such activity appears to be the down-regulation of host protective immune responses, and therefore enhance the ability of