Immunological Aspects of Urinary Schistosomiasis in Ibadan, Southwestern Nigeria

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SUMMARY

Humans infected with Schistosoma parasite demonstrate substantial immune responses against both the intravascular adult worms and the schistosoma eggs retained in the tissue. However, no immunologically mediated adverse effect on the adult worms has been described. Also, the strong cellular responses directed to the eggs are modulated during the course of chronic infection. Thus continued exposure of the host to worm and egg antigens usually lead to regulation of the engendered responses and maintenance of a stable chronic host-parasite relationship.This review aims at providing background information on the immunological status of Nigerian school children (especially in Ibadan) with urinary schistosomiasis.

History and Epidemiology

Schistosomiasis, also known as bilharziasis (after Theodor Bilhaz), is widespread with a relatively low mortality rate but a high morbidity rate, causing severe debilitating illness in millions of people [1].

Urinary schistosomiasis (caused by *Schistosoma haematobium*) was first discovered in soldiers of Napoleon stationed in Egypt between 1779 and 1891 who suffered severe haematuria [1]. In Nigeria, urinary schistosomiasis is known to have existed from time immemorial and might have been brought to the country by the migrating Fulani people when they travelled westwards from the Nile Basin [2]. The early record of urinary schistosomiasis in Nigeria is that of German explorer who, in 1881, published the occurrence of endemic haematuria in Bonny Province [2].

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A study carried out in the Department of Pathology, University College Hospital, (UCH), Ibadan, Nigeria between 1958 and 1963 revealed a relatively low incidence of pathological lesions due to schistosomiasis in Ibadan [3]. This must be accepted with caution because as at then, the incidence of *Schistosoma haematobium* ova in the urine of patients in UCH was only 3.1% [3].

According to W.H.O. report [1], Nigeria is one of the countries most seriously affected by urinary schistosomiasis and the disease is hyperendemic over large areas. Record survey in Ibadan showed that the infection is common among school children (above 5 years of age) especially males [4]. One of the reservoirs of urinary Schistosomiasis and its *Bulinus* snail intermediate host in Ibadan is an abandoned fish pond along Parliament Road, State Secretariat, Ibadan [5].

Immuology

Immune responses to an immunogen comprise cellular, humoral or innate immunity, but those that are evoked during *S. haematobium* infection are discussed below.

There is a dearth of information about immune responses to *Schistosoma haematobium* infection in Ibadan because earlier studies were concentrated on epidemiology and pathology. There is no sudden immunologic crisis leading to parasite elimination, but immunity develops gradually taking several years to become pronounced. In the early stage of infection, immunity is partial, nevertheless of vital importance in limiting the infection.

Innate Immunity

Innate immunity, which protects the body at all times from invasion by a vast majority of micro-organisms, may be natural, constitutional or genetic immunity. The skin is an example of innate immunological factor, which is known to protect against the invasion of cercaria.

The larva stage of *Schistosoma haematobium* that infect humans is the free-swimming, fork-tailed cercaria. Cercaria are attracted to the host by L-arginine and warmth [5]. Certain long chain poly-unsaturated free fatty acids percieved by argentophilic papillae of cercaria triggers its skin penetration and transformation to schistosomules. This transitional change must be accomplished within 10-15 minutes otherwise the cercaria will die off [5]. The implication of this is that the degree of infection tolerated by each host and skin susceptibility to lytic enzymes of the cercaria depend on the age of the host and the characteristics of the epidermal/dermal barrier of the skin. Thus, older hosts with enzyme-resistant polymerised skin are less susceptible to *Schistosoma* infection than very young host [5].

Involvement of genetic factors in the resistance to schistosomiasis is yet to be carried out in Nigeria. Genetic studies in Brazilians have shown that two distinct genetic loci control human susceptibility to schistosomiasis [26]. An interesting question to be answered is whether *Schistosoma* parasite has had any influence on the evolution of the mammalian im-mune system. The outcome will support the concept that infectious disease is the driving force for the polymophism observed in the major histocompatibility complex (MHC).

Immunoglobulin and Autoimmunity

The level and specificity of immunoglobulin in urinary schistosomiasis is controversial. This is due to variation in duration of infection, geographical origin of the host, intensity of infection and age of the patient [7]. Serum hypergammaglobulinaemia, hypoprotein-aemia and selective proteinuria are features of subjects with urinary schistosomiasis in Ibadan [8] as found in other environments [9].

Polyclonal B-cell activation found to be responsible for hypergammaglobulinaemia in urinary schistosomiasis 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 [10]. It is reasonable to propose that cytokines produced by Th2 subset of CD4+ cells play a crucial role in the induction of hypergammaglobu -

linemia. This hypothesis is supported by the well-known dependence of polyclonal IgE responses on IL-4 as well as a data suggesting a requirement for IL-6 in the induction of hypergammaglobulineamia [10].

The functional role of IgM and IgG4 in schistosomiasis was elucidated. IgM in urinary schistosomiasis acts as a blocking antibody while IgG4 controls antigen recognition by IgE and consequently regulates anaphylactic reaction and IgE mediated immunity [11]. Immunological pathways involving IgE have been shown to damage and protect against developing schistosomule but the slow build-up of protective IgE and early production of IgG4 blocking antibody are responsible for delaying the development of protective immunity against urinary schistosomiasis [13].

Levels of different classes of immunoglobulins vary at stages of urinary schistosomiasis. Serum IgA and IgM were found to be elevated at acute stage of urinary schistosomiasis [12], therefore suggesting the diagnostic use of these immunoglobulin classes.

Several protozoan infections are associated with extensive development of autoantibodies and, in a few documented instances, autoreactive T-cells. A data has implicated the involvement of *S. haematobium* parasite in the production of rheumatoid factor [24]. Nevertheless, an aetiological role of these autoantibodies in pathology (e.g. glomerulonephritis, anaemia) has not been directly established.

Complement Factors

The serum concentration of C3 is inconsistent (reduced or raised or normal) in patients with schistosomiasis [13]. In this environment (Ibadan), C3c was raised while C4 was reduced in children with urinary schistosomiasis [8]. The components of schistosomule glycocalyx have the ability to activate complement pathways to generate chemoattractants that promote adherence of phagocytic cells [14]. Despite the activation of complement system on the surface of schistosomule, it is not damaged or killed. How schistosomule survives complement activation is a mystery but may be associated with the existence of newly formed double

outer membrane. Warren [15] pointed out that as young worms develop and migrate to organs of the body, there are changes not only in their surface topography and morphology but also in their antigenicity.

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 [15]. Such antigens have been detected in serum and urine but these circulating antigen decrease with treatment and age of schistosomiasis patients [15]. 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 [16].

Clinical observations have shown that serum level of schistosomal antigen closely parallel the level of circulating immune complexes. The deposition of such immune complexes in basement membrane induces glomerular diseases [15,16].

T Lymphocyte - Mediated Pathology

Patients with urinary schistosomiasis develop a complex array of both humoral and cellular immune responses to soluble cercaria, worm and egg antigens, but only the egg antigens are important in the pathogenesis of granuloma disease [17].

Granulomas are localized cellular reactions against materials that are retained in tissues often for prolonged periods of time. They are complex inflammatory responses often classified as either "foreign body", or "hypersensitivity"-type granulomas. The former is primarily composed of macrophages and fibroblasts without an immunogenic nidus, while the latter occur in response to antigens and usually require T-cell sensitization. Most of the granulomatous pathology to schistosome egg belongs to the second category. These focal reactions represent the host's attempt to wall-off, contain, and perhaps destroy schistosome ova deposited in tissues (e.g. liver, intestine, bladder). Nevertheless, as lesions, which stimulate extensive tissue fibrosis, they are at the same time harmful to the host and are the primary cause of schistosomal disease [19,20].

The focal tissue destruction occurring in schistosomiasis, is followed by fibrosis, a wound-healing process in which fibroblasts are brought into the lesions and collagen is produced, resulting in scarring and obstruction of blood and/or lymphatic vessels. These events are clearly triggered by the inflammatory response to the parasite or its eggs and are dependent, at least in part, on T lymphocytes. In the case of schistosomiasis, the progressive fibrosis responsible for portal hypertension is thought to result primarily from egg granuloma formation [25]; while the two processes appear to be linked, they can be regulated differently.

In human schistosomiasis, Th1 cells produce high level of IFN and IL-2 at the pre-acute and acute stages of infection but production subsequently diminishes at chronic stage when IL-4 and IL-5 are, predominant [18]. A study in Nigeria (Ibadan) showed that immune responses controlled by Th2 cytokines predominate during urinary schistosomiasis though Th1- mediated immune responses are present. The implication is that diseases such as tuberculosis and leprosy that are resolved by Th 1 cytokines may be aggravated, while severe malaria and trichuriasis may be effectively controlled in subjects with urinary schistosomiasis [19]. This requires further investigation.

Mechanism of schistosomular killing by eosinophils and neutrophils

Complement components in the presence of antibodies are activated on the surface of schistosomula to attract eosinophils, which adhere via C3-C3 receptor interaction [20]. Following adherence eosinophils begin to flatten against the parasite surface so as to form an exact template of the surface topography. 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 of vacuolar contents onto the surface of the parasite [20]. Hydrolytic enzymes released by eosinophils are peroxidase, beta-glucuronidase, phospholipase-B, ribonuclease, aryl sulphatase and cathepsin, plus major basic protein from the granule crystalloid [21]. 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 [21]. Tegumental vacuolation follows and as a result of permeability changes in the remaining membrane, small lesions are formed through which eosinophil migrates [21]. After this, eosinophils flatten as they move between the tegument and the underlying muscle layers [20,21]. In this way, eosinophils strip the tegument away from the body of the worm.

A study showed that neutrophils are responsible for less damage of schistosomule when compared with the damage caused by eosinophils. This may account for the association of eosinophilia with urinary schistosomiasis [22]. Though a neutrophil possesses more 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 [23]. Neutrophil damages schistosomula by both direct physical means using pseudopodia, and enzyme action of the vacuoles. Neutrophils extrude enzymes (beta-glucuronidase, lactic dehydrogenase, myeloperodixase, and certain vasoactive amines) along the free border of the cells. After which, the adherent neutrophils push pseudopia processes into the tegument resulting in the death of the parasite [23].

Proposed Further Studies and Vaccine Strategies
Future work should relate rates of re-infection to age, sex, hormone levels, humoral and cellular immune responses, host genetics, and the incidence of other infections.

An effective vaccine against *Schistosoma* would be a valuable control tool and the high levels of protection elicited in rodents and primates by radiation-attenuated cercariae provide proof of principle. A major obstacle to vaccine development is the difficulty of identifying the antigens that mediate protection. The technologies collectively called proteomics, including 2D electrophoresis, liquid chromatography and mass spectrometry, now permit any protein to be identified provided there is extensive DNA data, and preferably a genome sequence. Applied to soluble (cytosolic) proteins from schistosomes, proteomics reveals the great similarity in composition between life cycle stages, with

several WHO vaccine candidates amongst the most abundant constituents. The proteomic approach has been successfully applied to identify the secretions used by cercaria to penetrate host skin, the gut secretions of adult worms and the proteins exposed on the tegument surface. Soluble proteins can also be separated by 2D electrophoresis before western blotting to identify the full range of antigenic targets present in a parasite preparation. The next step is to discover which target proteins represent the weak points in the worm's defences [27]. The vaccine study may be difficult to achieve in our environments because of compounding problems.

CONCLUSION
(i)   Certain functions of immune system (especially cell mediated immunity) in Nigerians with urinary schistosomiasis are adequate, this may account for the absence of severe malaria and viral infections in them.
(ii)   Based on immunological classification, urinary schistosomiasis in Nigerians is an acute stage disease.

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