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Evaluation of auto-antibody to DNA and soluble immune complexes in children with urinary schistosomiasis

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Summary

Levels of serum auto antibody to DNA and soluble immune complex (SIC) were determined in the sera of 40 Nigerian school children with heavy urinary schistosomiasis (USS), 64 school children with light USS and 36 apparently healthy children as controls. Haemagglutination and polyethylene glycol precipitation (PEG) techniques were employed respectively. 75% of USS subjects with heavy infection, 59% of USS subjects with light infection, and 8% of apparently healthy controls had auto antibodies to DNA in the serum samples. Moreso, the mean concentrations of auto-antibodies to DNA and SIC were significantly higher in USS subjects with heavy infection compared with USS subjects with light infection or the controls. Significant correlation existed between severity of *S. haematobium* infection (*S. haematobium* egg numbers/10ml urine) and SIC (r=0.21, t=2.16) or auto-anti DNA antibodies (r=0.43, t=3.86). It could be concluded that SIC and anti DNA antibodies are induced by antigens from eggs of *S. haematobium* and that both SIC and anti DNA antibodies may be involved in the pathology of USS.

Keywords: *Auto – antibodies, DNA, Immune complexes, schistosomiasis.*

Résumé

Les quantité d 'anticorps dans le serum d' DNA et le complexe d immunite soluble (SIC) etaient determinée dans le serum de 40 écoliers Nigerian avec une schistosomiase urinaire severe (USS), 64 ecoliers avec une legere schistosomiase urinaire et 36 ecolies apparemment sain utilize comme controle.Les techniques d'hémagglutination et de precipition polyethylene glycol(PEG) etaient utilisees.75% des subjests tres infectés d' USS et 59% des subjects legremenmt infectés and 8% apparemmnet sain avaient les auto anticorps contre l'DNA dans les echantillons de serum.Les concentrations moyennes d ' auto anticorps contre l DNA des subjects et SIC etaient significant et eleve chez les ecoliers ayant l USS grandement infectés comparé avec les ecoliers legerement infectés ou le group de controle.Il existe une correlation significant entre la severite de l infection au *S .hematobum* (nombre des oeufs de *s. hematobum*/ 10ml d urine) et SIC(r=0.21, t=2.16)or auto anticorps d anti DNA (r=0.43, t=3.86). Il pourrait etre conclu que le SIC et les anticorps contre DNA sont induit par les antigens des oeufs du *S.hematobum* et peuvent etre impliqué dans la pathologie de la schisstosomiase urinaire severe.

Introduction

Immune dysfunction is characteristic of chronic USS. The immunological changes in USS subjects include auto-immunity [1,2,3], raised immunoglobulin levels [4], decreased responses of lymphocytes to mitogens [5] and presence of serum factors which affects certain cell functions [6,7]. In addition, subjects with chronic schistosomiasis have an increased risk of other infections such as *Kleibsiella*, *E.coli*, *Proteus*, *Shigella* and *Salmonella* [8]. Studies in non-USS subjects attributed lymphocyte malfunction to DNA strand break [9], but normal DNA metabolism is fundamental to proliferation of cells and hence immune functions [9]. Apoptosis (programmed cell death which can manifest in DNA damage in cells) has also recently been described in USS subjects and in normal individuals [10]. Thus the levels of anti-DNA antibodies is expected to be raised in USS subjects. Though the presence of rheumatoid factor [11,12] and auto antibodies other than anti DNA antibodies [1,2,3] were reported in schistosomiasis patients but no study on human serum levels of anti-DNA antibodies was encountered. The present study evaluates and correlates serum anti-DNA antibodies and soluble circulating immune complexes in USS subjects to severity of *S. haematobium* infection.

Materials and method

The subjects:

The study was carried out on 104 Nigerian children with USS aged between 4 to 15 years (9.41±4.25 years). Forty (23 males and 17 females) were diagnosed as having heavy USS and 64 (40 males and 24 females) were diagnosed as having light USS. The diagnosis was carried out as previously described (4). Briefly, terminally spined eggs of *S. haematobium* were identified in the sediments of spun urine samples. *S. haematobium* infection was categorised into light- and heavy- infection based on the number of eggs counted per 10ml urine as described by W.H.O (13). *S. haematobium* egg count between 1 and 49 / 10ml urine was categorised as light infection while egg number of 50 and above was taken as heavy infection. A total of 36 S. *haematobium* free children and apparently healthy children were considered as controls. They were matched age, sex and socio-economic status.

Exclusion criteria:

Patients and controls with heavy infection of malaria, microfilaria, trypanosomiasis or intestinal helminths were excluded from the study. Malaria parasites (*Plasmodium*), *Trypanosoma* or micofilaria were examined in thick blood films stained with Giemsa stain. Intestinal helminthes infection was determined by examining characteristic eggs in normal saline preparation of faecal samples stained with Dobell iodine. Both faecal and blood samples were viewed under low power objective lens before confirmation with high power objective lens.

Assay for auto-antibodies to DNA.

Five ml blood was collected from each subject by venipuncture into plain bottle. The serum was separated after the blood had retracted at room temperature for 2hrs. Part of the serum was used immediately for the quantitation of SIC levels while the rest was stored at –20°C until required for auto-antibodies to DNA analysis. Antibodies to DNA in the subjects were detected by haemagglutination method using a test kit (Fujzoki, FD 304

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Japan) as described by the manufacturer. Twenty-five µl of serially diluted (1/5, 1/10, 1/20, 1/40, 1/80 and 1/160) inactivated serum samples from each of the subjects was put in U-bottomed plastic micro-titration trays. Seventy-five µl of diluted (1:11) and DNA un-sensitized red blood cells was added to the diluted test serum in wells containing 1:10 dilution of test serum while 75 µl of diluted (1:11) and DNA sensitized red blood cells was added to diluted test serum samples in wells containing 1/20-1/160 dilutions. Neither sensitized nor un-sensitized RBC was added to wells containing 1/5 dilution of test serum. The contents of the wells were gently but well mixed by shaking. This was covered with another tray on a sheet of white paper. Cell sediment in form of a button or ring with its edge shaping a smooth circle was regarded as negative. Smooth mat of cells covering the entire bottom of the reaction well but with edges folded was regarded as positive. Weakly reacting anti-DNA (1/160) were included as positive results. The reciprocal of agglutination end points were recorded.

Quantitation of soluble immune complexes:
Levels of SIC were assayed using the polyethylene glycol (PEG) precipitation method as previously described (4). PEG 6000 solution was added to serum in borate buffer to give a final concentration of 3.7% PEG and 1 in 3 dilution of serum. After incubation at room temperature for 1hr, the immune complex concentrations were measured at 450nm wave-length using spectrophotometer (MR 1001 plus).

Statistical analysis:
Statistical methods employed in the analysis of data generated in this study include: mean, standard deviation, Student's t-test, Chi-square and Pearson's correlaton co-efficient.

Results
Anti-DNA antibodies were detected in 75% of USS subjects with heavy *S. haematobium* infection, 59% of USS subjects with light infection and 8% of the controls respectively. The difference in positivity rates were significant (Table 1). Table 2 shows that the serum levels of SIC and anti-DNA antibodies were significantly higher in heavily infected USS subjects compared with either the lightly infected USS subjects or the controls.

Table 1: Comparison of the positive rates of anti-DNA antibody in the sera of USS subjects and the controls

	Total No. of subjects	Subjects with anti-DNA antibodies (+ ve)	Subjects with anti DNA antibodies (- ve)
Controls	36	3 (8)	33(92)
Light infection	64	38 (59)	26(41)
Heavy infection	40	30 (75)	10(25)

X^2 = 19.8. P < 0.0001 (s)
Percentages are in parentheses

Discussion
Antibodies to DNA was strictly used to investigate systemic lupus erythematosus pathogenesis, due to involvement of tissue injury and immune complexes in the disease [14]. However, anti-DNA antibodies have been reported in non auto-immune states such as chronic hepatitis [15] and malaria [16]. The detection of high titer of anti DNA antibody and serum SIC in USS subjects compared to the controls may be as a result of

Table 2: Levels of SIC and anti-DNA antibodies in USS subjects and the controls

	Heavy USS	Light USS	Controls
SIC (mg/dL)	23.00±5.2* n = 40	20.00±6.00* n = 64	12,00±2.3 n = 36
Anti-DNA antibody titer	0.180.02* n = 30	0.09±0.01* n = 38	0.01±0.006 n = 3

Values are means ± 1 S.D
**Significantly different from the controls (p value less than 0.05*
.Significantly different from light infection (p-value less than 0.05

accumulation and somatic mutation of immunoglobulins. Polyclonal B lymphocyte activation to produce excess IgM and IgA was earlier reported in Nigerian USS subjects [4].

Previous studies have shown that polyclonal activation of B lymphocytes participate in the development of auto-immunity [11,17]. Moreso, several kinds of auto anti-bodies are induced as a result of polyclonal B-lymphocyte activation after stimulation with lipopolysaccharides [18,19]. Therefore the occurrence of anti-DNA auto antibody may result at least in part from polyclonal activation of B lymphocytes by schistosome antigens. Also, the production of auto-antibodies could result from a break in self-tolerance. The B cell hyperactivity shown by the increase of anti DNA auto-anti-bodies and immunoglobulins [4] could be the consequence of defects in T- cell regulatory activities such as decrease of helper T cell functions, increased T suppressor cell activity as previously reported during schistosomiasis [21,22]. Schistosome derived mitogens was shown to cause polyclonal B cell activation [23]. Such components could explain the hyper-immunoglobulinaemia [4] and the production of rheumatoid factors [11] in USS.

Moreover, somatic mutations of these immunoglobulins might have increased their affinity for DNA to cause higher DNA-antiDNA auto-antibody complex which might have been detected as SIC. However, tissue damage/cell injury and inflammation as a result of *S. haematobium* infection is another alternative concept to explain higher concentration of anti-DNA antibody in USS subjects. All stages of schistosome parasites in human damage host tissues particularly during the migration of spiny eggs and schistosomules [24]. Red blood cells and white blood cells adhere onto the surfaces of adult schistosome worms and the eggs [24]. These blood cells are usually lysed [20] to release nuclear materials including DNA. This DNA is enzymatically degraded to oligonucleotides by plasma DNAase. Small chain nucleotides derived from DNAase activity may not be cleared from the circulation as rapidly as larger molecules, thus stimulate anti-DNA antibody production. The sizes of immune complex determine its fate, while large, relatively insoluble complexes are rapidly removed by the reticulo-endothelial system, low molecular weight complexes are removed more slowly. Thus, preponderance of low molecular weight DNA/anti-DNA complexes may be present in USS subjects. Low molecular weight immune complexes usually persist in the circulation but under appropriate conditions of vascular permeability, these soluble immune complexes may be deposited in the blood vessel walls or basement membrane of the glomerulus, thereby activate complement components and thus produce vascular/glomerular damage. Glomerulonephritis, proteinuria, inflammation and tissue damage are com-

mon to USS [7,8] and certain auto immune states [21]. However, investigation into the exact mechanism by which anti-DNA antibody participate in the induction of glomerulonephritis is yet to be concluded.

The detection of anti-DNA antibody in 2% of the control children supports previous findings [11,22] where rheumatoid factor (another index of auto-immunity) was detected in apparently healthy Nigerians. Normally, unwanted cells are damaged in the body by apoptosis and the action of endogenous endonuclease/ DNAase could lead to the production of oligonucleotides and subsequently elicit anti-DNA antibody in individuals with no apparent infections.

There are several ways by which a schistosome infection could cause immuno-depression. Among them is activation induced apoptosis which provide a mechanism for the elimination of schistosome-reactive T cells [10]. Nuclear products of apoptotic T-lymphocytes may stimulate immune response for anti DNA antibody production. In systemic lupus erythematosus patients, Lewis and Roberts [21] postulated that nucleosomes, which are generated by cellular apoptosis stimulate nucleosome specific antibodies. These antibodies serve as a bridge between various anti-nuclear auto-antibodies and the negatively charged heparan sulphate, which is an intrinsic constituent of the glomerular basement membrane. Similar mechanism could be conjectured in USS.

This study suggests that production of anti DNA auto antibodies and SIC may be involved in the immuno-suppression in USS subjects.

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