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IMMUNE COMPLEXES IN SCHISTOSOMIASIS

VI — Circulating IC levels in patients with and without nephropathy

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SUMMARY

Circulating immune complexes (CIC) were investigated by the 125I-C1q binding test in serum from schistosomiasis patients with and without renal disease. The mean level of CIC in serum from patients without nephropathy was weakly higher than that from patients with renal injury. Similar amount of CIC was found in different types of glomerular lesions (membranous proliferative glomerulonephritis and focal glomerular sclerosis). Immunoglobulins G and M, C3 component of complement and fibrin were demonstrated in renal glomerular deposits from patients with and without nephropathy. The involvement of CIC in schistosomal nephropathy is discussed.

INTRODUCTION

Glomerular lesions are pathological findings generally associated with the deposition of antigen-antibody complexes. In human schistosomiasis, a chronic scleroting glomerulonephritis, intially described in post-mortem studies [1,15] was also observed in large series of renal biopsy, which showed a spectrum of glomerulonephritis mainly in hepatosplenic patients with or without renal disease [6,20,21]. Granular deposits of IgG, IgM and C_3 [13,27] and recently of schistosomal antigen [12] were found in some human glomerular lesions. Similar deposits were also demonstrated in mice and hamsters infected with **S. mansoni** [12,18].

Circulating schistosome antigens have been found in serum and urine from infected animals [2,3,9,11,14,17]. In human infection, specific schistosome antigens were demonstrated in urine [7,19] and milk [22,26]. All these findings support the detection of circulating immune complexes (CIC) in human [4,5,24,25] and murine schistosomiasis [4,25].

In this work we attempt to correlate CIC levels in schistosomiasis patients with and without renal disease, with the type of glomerular lesions, and glomerular deposits of immunoglobulin, C_3 and fibrin.

MATERIAL AND METHODS

The diagnosis of schistosomiasis was based upon the clinical manifestation and epidemiological characteristics of the case and was confirmed by identification of viable eggs of **S. mansoni** in the stool. Seventeen patients were studied through histological, immunopathological and CIC determinations. Five of them (group I) without renal disease, were biopsied during the splenectomy and in twelve (group II) with renal disease, percutaneous renal biopsy were performed.

For light microscopy, tissue was fixed in Bouin's solution, cut at 2 to 3 microns and stained with hematoxylin and eosin, periodic acid schiff, periodic acid methanamine silver and Heidenhain's connective tissue stain.

For immunofluorescence studies, renal fragments were snapfrozen in liquid nitrogen

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and cut in a cryostat at -25°C. Each section rapidly fixed in acetone, was washed in buffered saline, pH 7.2, covered with fluorescein-labeled antiserum, washed three times in buffered saline and mounted with buffered glycerin. Specific fluorescein antisera to IgG, IgM, C3 and fibrin, obtained from Hyland Laboratories (Costa Mesa, Ca), were previously tested, diluted and used in five cases.

The 125I-C1q binding test was used for the detection of CIC in patients' sera [4,28]. Briefly, C1q was isolated from normal human serum and labeled with 125I. This preparation was then mixed with test serum, previously treated with 0.2 M EDTA. Free 125I-C1q was separated from 125I-C1q bound to complexes by precipitation with 3% polyethylene glycol.

Results were expressed in per cent 125I-C1q precipitated as compared with the protein-bound radioactivity precipitable with 20% trichloracetic acid. The level of CIC was considered significant starting from 13% [24].

Levels of C3, C4 and C1q were quantified in patients' sera by radial immunodiffusion [16] on plates supplied by the Behring Institute (Marburg, Lahn, W. Germany).

Statistical analysis was done by the chi-square test.

RESULTS

Histological studies with light microscopy are shown in Table I. The group I, patients with normal renal function, showed minimal histopathological changes on the kidney biopsy, characterized by mesangial matrix increase without hypercellularity, swollen endothelial and epithelial cells, and proteinaceous material of the Bowmann's space. In the group II (Table I), all patients had a nephrotic syndrome, except in one case (patient M. S.C.) with proteinuria only. In this one, a mild proliferative glomerulonephritis was seen. Three patients had a membranous proliferative glomerulonephritis (Fig. 1), with mesangial hypercellularity and splitting of basement membrane, and two of them (patients A.T. and S.J.), with lobular accentuation (lobular type). Eight patients had a focal segmental glomerular lesion, with mild mesangial hypercellularity, mesangial sclerosis, collapse of peripheral capillary loops and adhesions to Bowmann's capsule (Fig. 2).

TABLE I

Circulating immune complexes (CIC) in schistosomiasis patients with and without nephropathy

Group	Patients	Clinical form	Glomerular lesions	CIC (% ^{125}I-C1q precipitation)	
I	J.C.B.	HS	Minimal histopathological changes	26%	
	M.E.B.	HS	Minimal histopathological changes	17%	
	R.L.B.	HS	Minimal histopathological changes	13%	mean ± s.d.
	A.A.B.	HS	Minimal histopathological changes	18%	18.6 ± 4.7
	D.A.	HS	Minimal histopathological changes	19%	
II	M.S.C.	IS	Proliferative G.N. Gen. Difuse	14%	
	M.A.	HS	Membranous proliferative G.N. Gen. Segmental	12%	
	A.T.	HS	Membranous proliferative G.N. Gen. Difuse	18%	
	S.J.	HS	Membranous proliferative G.N. Gen. Difuse	14%	
	H.E.	IS	Focal Glomerular Sclerosis	14%	
	L.R.	HIS	Focal Glomerular Sclerosis	9%	mean ± s.d.
	R.C.	HIS	Focal Glomerular Sclerosis	18%	14.2 ± 3.2
	J.C.A.	HIS	Focal Glomerular Sclerosis	12%	
	M.S.B.	HS	Focal Glomerular Sclerosis	20%	
	S.R.	HS	Focal Glomerular Sclerosis	13%	
	M.J.	HS	Focal Glomerular Sclerosis	11%	
	S.C.	HS	Focal Glomerular Sclerosis	16%	

IS — Intersticial Schistosomiasis
HIS — Hepato-intestinal Schistosomiasis
HS — Hepatosplenic Schistosomiasis

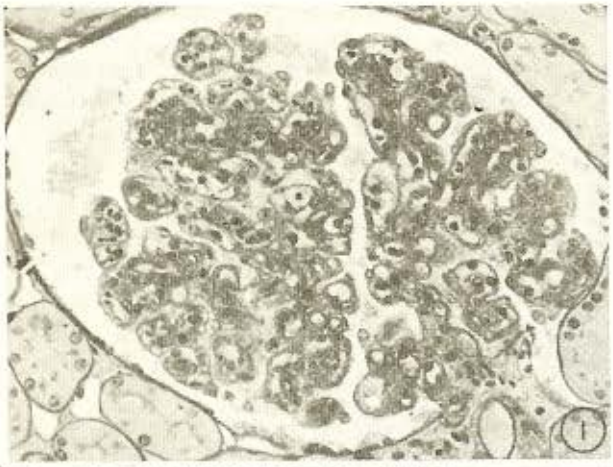

Fig. 1 — Membranous proliferative glomerulonephritis in schistosomiasis mansoni: mesangial cells hyperplasia with hyperlobulation and splitting of the glomerular basement membrane. PAS, 250X

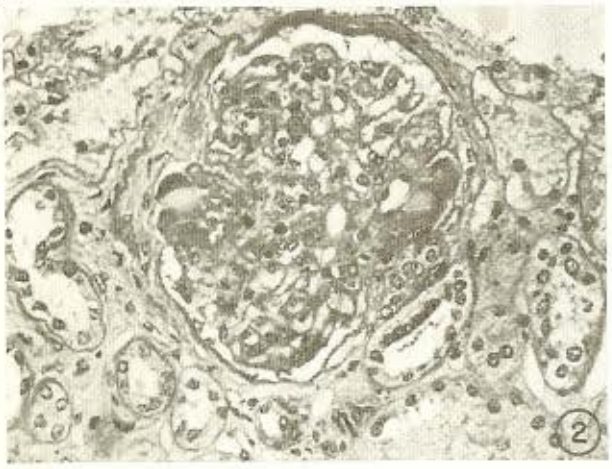

Fig. 2 — Focal glomerular sclerosis in schistosomiasis mansoni: segmental hypercellularity with mesangial sclerosis, adhesions to the thickened Bowmann's capsula and subendothelial hyaline deposits. PAS, 250X

Concerning CIC (Table I), the mean level of 125I-C1q binding in serum from group I patients (18.6 ± 4.7), was weakly higher ($P < 0.05$) than that from group II (14.2 ± 3.2). CIC levels were not different in patients with membranous proliferative glomerulonephritis (14.5 ± 2.5) and patients with focal glomerular sclerosis (14.1 ± 3.7).

TABLE II

Circulating immune complexes (CIC) and deposits on the glomerular lesions

		Granular deposits				CIC	C3	C1q	C4
Patients	Glomerular lesions	IgG	IgM	C3	Fibrin	%^{125}I-C1q ppt.	mg%ml	mg%ml	mg%ml
D.A.*	M.H.C.	++	++	++	+	19%	110	15	35
M.A.*	M.P.G.M.	++	+	++	0	12%	97	15	14
H.E.	F.G.E.	++	++	+++	++	14%	158	21.5	27
R.C.	F.G.E.	++	+	++	0	18%	154	7	27
M.J.	F.G.E.	++	+	+++	0	11%	175	22	35

M.H.C. — Minimal Histopathological changes
M.P.G.N. — Membranous Proliferative Glomerulonephritis
F.G.E. — Focal Glomerular Sclerosis
* — Tubular basement membrane and intersticial deposits of IgG

Results obtained in immunofluorescence are given in Table II. Four patients with renal disease revealed minimal to mild granular deposits of IgG (Figs. 3 and 4), IgM and C3 in all cases, and fibrin in one case (patient H. E.). One patient (D.A.), without renal disease, also showed glomerular deposits of IgG, IgM, C3 and fibrin. In two circumstances (patients D.A. and M.A.), deposits of IgG also were present in interstitial and tubular basement membrane. No correlation was observed between glomerular deposits and levels of CIC, C3 C4 and C1q in serum (Table II).

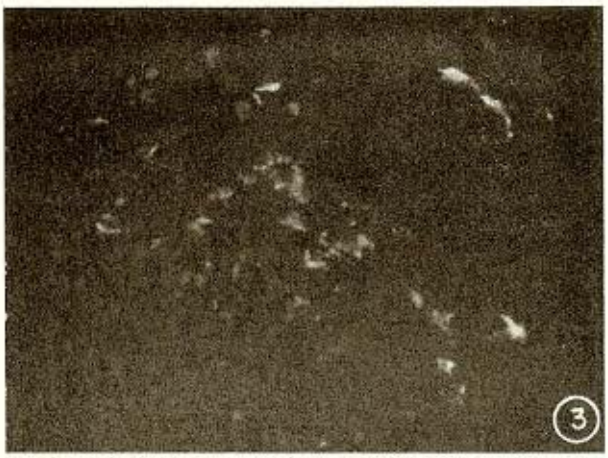

Fig. 3 — Direct immunofluorescence on kidney biopsy material from a membranous proliferative glomerulonephritis schistosomiasis patient: granular deposits of human IgG in peripheral capillary loops and mesangial areas. 250 X

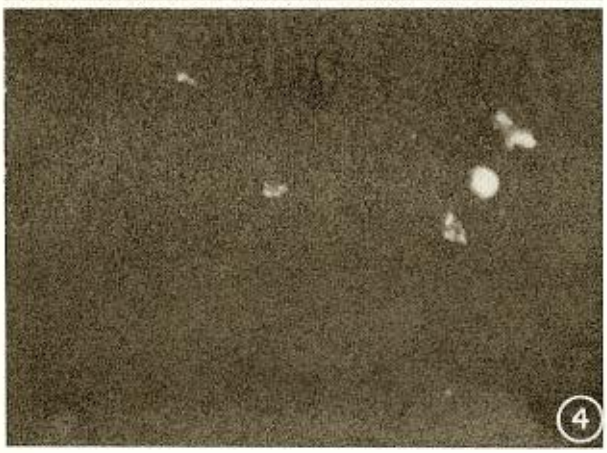

Fig. 4 — Direct immunofluorescence on kidney biopsy material from a focal glomerular sclerosis schistosomiasis patient: segmental space bumpy deposits of human IgG. 250 X

DISCUSSION

The present study shows, in human schistosomiasis, that CIC levels do not differ significantly between patients with and without renal disease. The biological significance of this result should be considered in several points. The involvement of antigen-antibody complexes in the renal injury associated with **S. mansoni** schistosomiasis, particularly the hepatosplenic form of the disease, has long been suspected [1,6,15,20,21]. Glomerular granular deposits of IgG, IgM, C3 and fibrin, demonstrated in this study confirms several works previously described in human schistosomiasis [13,27]. All these findings associated with the detection of schistosomal antigen in renal glomeruli [10,13] suggest strongly the involvement of immune complexes in schistosomal nephropathy. If the participation of antigen-antibody complexes in the schistosomal glomerular lesions is a likely event, the site of their formation is, until now, unknown. Two possibilities may occur. Firstly, these complexes are formed in the blood circulation. The detection of CIC in serum from patients

infected with **S. mansoni** [4,5,24,25] confirms this possibility. Small amounts of these CIC would be fixed into vessel walls or filtering membranes such as in renal glomeruli [8]. However, the little difference, obtained in this work, in the CIC levels between patients with or without nephropathy do not allow to suggest the deposition of these CIC into the kidney. Alternatively, if the antigen-antibody complexes are formed in renal glomeruli, large molecules of uncomplexed schistosomal antigens or CIC in large antigen excess, derived from circulating blood, could be retained in glomerulus. After passage of corresponding antibodies, the complexes would be formed in antibody excess. These "glomeruli-formed immune complexes" after complement activation, would be the major factor of the characteristic inflammation of schistosomal nephropathy. The involvement of complement components in immune complexes detected in serum from patients infected with **S. mansoni** [23] is an additional argument against the deposition of CIC. In fact, if CIC have activated and fixed the complement before their deposition, the complement-dependent activation inflammatory phenomena of schistosomal glomerular injury, would be more difficult to occur.

Similar amount of CIC was found in serum from patients with different types of glomerular lesions. Glomerular granular deposits of immunoglobulins, C3 and fibrin were also found in all the types of studied glomerular injury. These findings might suggest that antigens-antibody complexes are implicated in various schistosomal glomerular pathology. The composition and the size of deposited immune complexes could be one of the parameters associated with the different types of glomerular lesions. These **S. mansoni** deposited complexes could explain the maintainance of mesangial hypercellularity, present in any type of schistosomal glomerular injury, independent of the clinical form of schistosomiasis [6,20]. Further investigations on the origin of the formation site of the immune complexes involved in schistosomal nephropathy are underway.

RESUMO

Imuno-complexos na esquistossomose. VI — Dosagem dos IC circulantes em pacientes com ou sem nefropatia

Os imuno-complexos circulantes (CIC) foram investigados pelo teste de ligação ao C1q-I125 no soro de esquistossomóticos apresentando ou não uma lesão renal. A taxa média dos CIC foi fracamente superior nos doentes sem nefropatia que nos esquistossomóticos com lesão glomerular. Nenhuma diferença nas taxas de CIC foi observada entre os dois tipos de lesões estudados (glomerulonefrite membrano-proliferativa e esclerose glomerular focal). As imunoglobulinas G e M, a fração C3 do complemento e a fibrina foram caracterizadas nos depósitos glomerulares renais dos doentes com ou sem uma nefropatia. O papel representado pelos CIC na nefropatia esquistossomótica é analisado.

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