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Immunoglobulin-A and the pathogenesis of schistosomal glomerulopathy

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Immunoglobin-A and the pathogenesis of schistosomal glomerulopathy. Several observations suggest that the evolution of schistosomal glomerulopathy into clinically overt and progressive disease may involve pathogenetic mechanisms other than simple glomerular deposition of parasitic antigens. In a previous study, IgA was suggested to be a mediator of late glomerular lesions in this disease. This issue is further addressed in this work. The study includes 32 patients with hepatosplenic schistosomiasis, of whom 16 had overt glomerular involvement, along with four control groups: (a) 15 healthy volunteers; (b) 15 patients with simple intestinal mansoniasis; (c) 17 patients with non-schistosomal chronic liver disease; and (d) 21 subjects with primary nephrotic syndrome not associated with schistosomiasis. Routine assessment was done for all subjects including confirmatory tests for schistosomal infection, liver and renal function tests, hepatitis viral markers and abdominal ultrasonography. The total serum concentrations of IgG, IgM, IgA were measured, as well as their respective circulating immune complexes, rheumatoid factors, anti-gliadin- and anti-DNA-antibodies. Liver and renal biopsies were obtained from the relevant groups and studied by light microscopy. Renal biopsies were also examined by immunofluorescence. Patients with simple intestinal schistosomiasis had a significant increase in IgM antigliadin antibodies. Those complicated with hepatosplenic involvement also had a significant increase in the mean IgG anti-gliadin antibodies, IgG rheumatoid factor and IgM anti-DNA activity. Cases further complicated by overt glomerular disease showed a distinct IgA predominance, mainly expressed in the serum anti-gliadin antibody pool and anti-DNA activity. This profile was essentially similar to that observed in control cirrhotics. There was a significant increase in the frequency of IgA glomerular deposits in renal biopsies obtained from patients with overt schistosomal glomerulopathy, in contrast to control nephrotics. The deposits were mainly mesangial, but were also encountered in subendothelial, subepithelial and peritubular locations. Their frequency was significantly higher with more advanced lesions as seen by light microscopy. The relevance of these data is discussed, leading to the following conclusions: (a) serum IgA-anti-gliadin and -anti-DNA antibodies, and glomerular IgA deposits are markers of significant renal involvement in patients with hepatosplenic schistosomiasis. (b) IgA may be involved in the pathogenesis of advanced glomerular pathology when superimposed on parasite-induced lesions. (c) There is a significant increase in serum auto-reactivity in hepatosplenic schistosomiasis, which may also have pathogentic implications. (d) Increased production by the inflammatory bowel lesions, impaired clearance by the fibrotic livers and probable switching of immunoglobulin synthesis are suggested to explain the observed IgA predominance in those who develop renal complications.

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Glomerulonephritis often complicates chronic parasitic infestations as schistosomiasis, malaria, leishmaniasis, filariasis, echinococcosis, trichinosis, trypanosomiasis and others [1]. The brunt of glomerular injury is usually mesangial, expressed as cellular proliferation and matrix expansion in a diffuse or focal pattern [1]. Immune complexes comprising parasitic antigens [2–5] and specific antibodies [6], mostly composed of IgM, are usually detected in the early stages of glomerular pathology. In the majority of cases, such lesions are either subclinical [7], transient [8, 9] or mild enough to be overshadowed by the prominent manifestations of the original illness [10]. Schistosomiasis is one of the few notable exceptions in that it is often associated with overt features of glomerular disease including the nephrotic syndrome [11], hypertension [12], urinary abnormalities [13], and progression to chronic renal failure [14].

The clinicopathologic spectrum of schistosomal glomerulopathy is probably the widest among parasitic nephropathies, ranging from silent mesangial proliferation to glomerulosclerosis [15]. Little is known about the factors leading to the selection of some 12 to 15% of patients with hepatosplenic schistosomiasis who progress to clinically overt disease, and further on to ESRD [16]. Certain agent factors are often incriminated, including the parasite's species and strain, duration of the disease, and probably certain associated infections. Host factors may even be more important, including genetic influence, autoimmune reactivity, and hepatic fibrosis [16]. The latter has attracted the attention of students in the field over the past three decades, on the basis of experimental [17–19], clinical [12] and post-mortem [20] observations. The sovereignty of IgA mesangial deposits in late clinical [21] and experimental [22] schistosomal glomerulopathy suggests that this immunoglobulin may be an important mediator of the "liver effect" in schistosomal glomerulopathy.

This work was designed to examine such conjecture through studying the principal forms of circulating IgA in clinically overt schistosomal glomerulopathy in comparison with appropriately selected control groups.

Method

The study includes 101 subjects, comprising two target cohorts and four control groups (Table 1). The former (Groups I and II) are composed of all consecutive patients with hepatosplenic schistosomiasis presenting to the Out-patient clinics of Cairo University or the Theodore Bilharz Institute hospitals over one

Table 1. Study population

Group	Title	Subjects included	Age *years* Range	Age *years* Mean ± SD	Female/Male	Description
I	Hepatosplenic schistosomiasi without proteinuria	16	13–40	29.3 ± 9.03	2/14	Biopsy-confirmed hepatosplenic schistosomiasis without dip-stick detectable protein on 3 occasions in morning and post-exercise mid-stream urine samples
II	Hepatosplenic schistosomiasis with glomerulopathy	17	13–48	34.2 ± 8.67	4/13	Biopsy-confirmed hepatosplenic schistosomiasis with clinicopathologic evidence of schistosomal glomerulopathy[a]
III	Healthy controls	15	27–36	31.0 ± 2.75	5/10	Healthy volunteers from medical and paramedical staff
IV	Simple schisto	15	13–32	21.0 ± 5.66	0/15	Uncomplicated *S. mansoni* intestinal schistosomiasis
V	Non-schistosomal chronic liver disease	17	30–52	39.8 ± 6.16	1/16	No evidence of previous or current infection[a]
VI	Primary nephrotic syndrome	21	15–33	23.2 ± 5.4	8/13	No evidence of previous or current infection (Table 2).
		101			20/81	

[a] Details in text

Table 2. Histopathological diagnosis in subjects with primary nephrotic syndrome

Mesangioproliferative glomerulonephritis	9
Mesangiocapillary glomerulonephritis	6
Minimal change disease	2
Amyloidosis	2
Membranous nephropathy	1
Focal & segmental glomerulosclerosis	1
Total	21

year. The diagnosis was based on: (1) clinical and ultrasonographic [23] evidence of hepatosplenic schistosomiasis; (2) histopathologic evidence of periportal fibrosis sparing the lobular architecture, with or without schistosomal ova or pigments [24]; and (3) detection of *S. mansoni* ova in the stools or rectal snips. Group I cases had no dip-stick detectable proteinuria (Table 1), while those included in Group II had overt proteinuria, with renal biopsy findings compatible with schistosomal glomerulopathy [15].

Exclusion criteria comprised: (1) evidence of associated urinary schistosomiasis (hematobiasis); (2) clinical, serological or histopathological evidence of associated liver disease, such as viral hepatitis; (3) evidence of other secondary glomerulopathy; (4) impaired hepatocellular function; and (5) serum creatinine above 2 mg/dl.

The control groups (Table 1) comprised: (a) selected healthy volunteers from the medical and paramedical staff (Group III); (b) randomly selected patients with confirmed uncomplicated intestinal schistosomiasis (Group IV); (c) randomly selected patients with non-schistosomal chronic viral hepatic disease including "chronic persistent" hepatitis (3 cases), "chronic active" hepatitis (6 cases), "active cirrhosis" (4 cases), and established post-hepatitic cirrhosis (4 cases) (Group V); and (d) contemporary patients with primary glomerulonephritis subjected to routine renal biopsy (Table 2), provided they had no evidence of past or present schistosomal infestation (Group VI).

All subjects were clinically assessed, and subjected to routine laboratory evaluation by standard methodology, which included: (1) search for evidence of schistosomal infestation by examination of the urinary sediment, stools analysis and circum-oval precipitin test. (2) Determination of serum bilirubin, AST and ALT. (3) Determination of the serum total protein concentration and the electrophoretic pattern. (4) Testing the serum reactivity for markers of HBV and HCV. (5) Routine urine examination and determination of 24-hour urinary protein. (6) Determination of BUN and endogenous 24-hour creatinine clearance. (7) Ultrasonic examination of the abdomen.

The serum profiles of immunogloblins G,A and M were studied using the following methodology.

Total serum concentrations

These were measured by an ELISA assay [25].

Plates (Nunc Immunoplate I, Roskilde, Denmark) were coated overnight at +4°C with polyclonal IgG of goat anti-human α, μ or γ chain (CAPPEL Cochranville P.A.) at a concentration of 100 μg/ml in 0.1 M carbonate buffer pH 9.6. Plates were washed with Titertek Microplate Washer (Flow Laboratories, Irvine, UK). Sera diluted in 0.05 M phosphate buffer pH 7.3 containing 0.05% Tween 20 and 0.1% BSA were deposed in triplicate (1/150000 for IgA, 1/10000 for IgM, 1/400000 for IgG). After one hour at 37°C, plates were washed. One hundred microliters of peroxidase conjugated polyclonal IgG goat anti-human α, μ or γ chain diluted at optimal concentration in phosphate buffer were added in each well. After incubation and washing, 100 μl of substrate consisting of 4 mM orthophenylenediamine (Sigma Chemical Co., St. Louis, MO, USA) in 0.02 M citrate phosphate buffer pH 5.0 containing 0.04% (vol/vol) H_2O_2 were added. Color development was stopped after 10 minutes with 50 μl HCl 1 N. Absorbance of each well was read at 492 nm with a Titertek Multiscan (Flow Laboratories). IgA, IgG and IgM concentrations were evaluated according to a standard curve. Results were expressed in grams per ml.

Circulating immune complexes

Levels of circulating immune complexes (CIC) containing C3 and IgA, IgG or IgM were estimated by a solid-phase enzyme-linked immunosorbent assay [26–29].

Aggregated immunoglobulins. IgA from colostrum or human IgG were aggregated at a concentration of 10 mg/ml by heating at 63°C for 150 minutes. Bovine serum albumin (fraction V; Sigma) was added to all preparations at a final concentration of 0.5% in order to stabilize the composition of the aggregated immunoglobulins. Aggregated IgM were purified from the serum of a patient with

IgM myeloma by a chromatography on Sephacryl S 300 (Pharmacia Sweden). Before use, aggregated IgA and IgG were preincubated for one hour at 37°C with a pool of normal human sera in order to fix iC3b [29] and then treated as tested sera.

PEG precipitation. Immune complexes were precipitated from the sera with PEG 6000. Fifty milliliters of serum were added to 1 ml of 3.8% PEG 6000 (OSI, Paris, France) in phosphate buffer saline pH 7. After 90 minutes in ice, the precipitates were centrifuged at 2400 g for 20 minutes and then washed with 1 ml of 3.5% PEG. After centrifugation, the precipitates were redissolved in 250 ml of 0.05 M phosphate buffer, pH 7.3, containing 0.05% Tween 20 and 0.1% BSA.

ELISA assay. Plates were coated overnight with F(ab') 2 fragments of goat IgG antihuman C3 (Capell, Cochranville, PA, USA) diluted to 10 mg/ml in 0.1 M carbonate buffer, pH 9.6. One hundred milliliter aliquots of redissolved PEG precipitates were added to anti-C3 coated wells at a dilution equivalent to 1/100 for CIC IgA or IgG and 1/200 for CIC IgM of the original serum. After one hour at 37°C, plates were washed and 100 ml of peroxidase conjugated F (ab') 2 fragments goat anti-human α, μ or γ chain (Cappel) were added. Reactions were ended as described in RF assay (described below). Results were expressed as a percent of aggregated immunoglobulins.

Serum auto-reactivity

This was assessed by the assay of anti-DNA antibodies and rheumatoid factor activity.

Anti-DNA antibodies. Samples were screened for the presence of anti-DNA antibodies by an enzyme-linked immunosorbent assay [30].

Plates were coated overnight at +4°C with native DNA from calf thymus (Sigma) at a concentration of 25 mg/ml in 0.1 M carbonate buffer pH 9.6. After washing, free sites were blocked by incubation with a 0.2% gelatin solution in carbonate buffer during one hour at 37°C. Sera diluted at 1/100 for IgA and 1/800 for IgG and IgM were deposed in triplicate and incubated one hour at 37°C. Then 100 ml of peroxidase conjugated IgG goat anti-human α, μ or γ chain were added to each well. After washing, OPD was added and resulting optical density was determined at 492 nm (c.f. RF assay). Results were expressed as an index.

Rheumatoid factor activity. IgA, IgG and IgM rheumatoid factors (RF) were detected by an enzyme-linked immunoassay [28, 29, 31].

Plates were coated with non-aggregated human IgG (Sigma) at a concentration of 10 mg/ml in 0.1 M carbonate buffer, pH 9.6, for IgM and IgA RF and with non-aggregated horse IgG at 10 mg/ml for IgG rheumatoid factor. After a night at +4°C, the plates were washed with saline buffer containing 0.05% Tween 20. Sera diluted at 1/100 in 0.05 M phosphate buffer, pH 7.3, containing 0.05% Tween 20 and 0.1% BSA for RF IgA and at 1/400 for FR IgG and FR IgM were deposed in triplicate. After one hour at 37°C, the plates were washed and 100 ml of peroxidase conjugated F(ab') 2 fragments, goat anti-human α, μ or γ chain (Cappel) appropriately diluted were added to each well. Plates were incubated one hour at 37°C and then exposed to 100 ml of 4 mM OPD in 0.02 M citrate phosphate buffer pH 5.0 containing 0.04% (vol/vol) H_2O_2). After HCL addition, absorbance of each well was read at 492 nm. Sera were tested in the presence of a pool of normal sera and a pool of sera containing rheumatoid factor for each class of immunoglobulin. Results were expressed as an index.

Anti-gliadin antibody assay

This was chosen as a parameter of the intestinal mucosal immunoreactivity to food antigens. Their value as a marker of disease activity in chronic inflammatory bowel disease is confirmed [32].

Antigliadin antibodies were measured by an ELISA technique [32]. Plates were coated with a gliadin at 10 mg/ml in carbonate buffer, pH 9.6, overnight at +4°C. Sera diluted at 1/50 for IgA, 1/100 for IgG, and 1/200 for IgM were deposed in triplicate. After one hour at 37°C, plates were washed and peroxidase conjugated goat IgG anti-human α, μ or γ chain were added. After incubation (1 hour at 37°C), 100 ml of substrate (OPD as for DNA assay) were added for 10 minutes at room temperature. Color development was stopped with 50 ml HCl 1 N. All sera were tested in the presence of a pool of normal sera and a pool of sera containing IgA, IgG, or IgM anti-gliadin. Results were expressed as an index.

For all these methods, the sera were tested in the presence of positive sera and negative sera. Positivity threshold was calculated from the results of 14 patients belonging to Group V, being defined as mean +2 standard deviation.

Unless contra-indicated, a percutaneous liver biopsy was obtained from Groups I, II and V. Paraffin sections were prepared, stained by hematoxylin and eosin and orsin stains, and examined by light microscopy.

Percutaneous renal biopsy was obtained from Groups II and VI. Paraffin sections were prepared, stained by hematoxylin and eosin, PAS, Masson trichrome, silver and Congo red stains, and examined by light microscopy and when necessary under polarized light. Frozen sections were treated by fluorescin-labeled antisera for IgG, IgM, IgA, C3, fibrinogen and control albumin, and examined by immunofluorescence.

All data were subjected to statistical analysis using Student's *t*-test, paired *t*-test and analysis of variance.

Results

The two target groups, (Groups I and II) were neatly matching as regards age and gender distribution, clinical parameters and liver functions. Patients with associated glomerulopathy (Group II) had proteinuria ranging from 0.2 to 10.0 (mean 5.2, SD 3.16) g/24 hrs; 11.7% were hypertensive, and their creatinine clearances ranged from 39.2 to 106.8 ml/min with a mean of 73.74 ± 25.00 ml/min compared to 103.4 ± 24.24 ml/min in those without dip-stick detectable proteinuria ($P < 0.001$).

They also generally matched with the control groups as regards age and gender. Yet, patients with uncomplicated intestinal schistosomiasis and those with primary nephrotic syndrome were significantly younger, and the relative contribution of females was higher in the latter group (Table 1). Group V (non-schistosomal chronic liver disease) had significantly higher serum bilirubin (mean 1.52 ± 1.71 mg/dl compared to 0.68 ± 0.22 mg/dl and 0.76 ± 0.38 mg/dl in Groups I and II, respectively) and hepatic transaminases (AST 31.05 ± 19.84 IU/liter compared to 16.56 ± 7.08 and 17.00 ± 12.07 IU/liter, respectively; ALT 30.52 ± 20.03 IU/liter compared to 12.68 ± 5.64 and 13.64 ± 6.39 IU/liter, respectively). None of Group V patients was hypertensive or proteinuric, and their renal function was normal (mean creatinine clearance 91.67 ml/min). Group VI (those with primary nephrotic syndrome) had a marginally higher protein excretion compared to Group II cases (6.7 ± 4.8 vs. 5.2 ± 3.16 g/24 hr, respectively). They

Table 3. Concentrations and proportional contributions of total serum immunoglobulins, circulating immune complexes and rheumatoid factors

	Group I			Group II			Group III		Group IV			Group V			Group VI		
	Mean	SD	*P*	Mean	SD	*P*	Mean	SD	Mean	SD	*P*	Mean	SD	*P*	Mean	SD	*P*
Total serum concentration *g/liter*																	
IgA	3.07	0.81		3.53	1.43	< 0.030	2.72	0.60	2.76	0.53		3.76	1.13	< 0.002	2.92	0.88	
IgG	24.13	2.52	< 0.001	21.47	8.19		19.24	3.20	20.08	2.82		24.78	3.38	< 0.001	17.95	4.71	
IgM	2.96	0.61	< 0.001	2.96	0.53	< 0.001	2.38	0.29	2.61	0.54		3.09	0.60	< 0.001	3.05	94.00	< 0.006
Circulating immune complexes %																	
IgA	25.68	19.55	< 0.006	29.73	22.09	< 0.002	11.26	6.51	12.20	6.02		35.93	22.37	< 0.001	15.71	8.35	< 0.050
IgG	37.25	20.50	< 0.001	34.53	26.80	< 0.010	16.86	6.88	15.86	5.69		43.68	26.95	< 0.001	18.47	14.14	
IgM	26.56	11.48	< 0.009	24.20	12.69	< 0.010	15.40	5.24	17.66	7.81		28.50	18.56	< 0.007	19.95	10.71	
Rheumatoid factors (Index)																	
IgA	19.62	12.98		23.33	14.92		17.13	11.80	11.80	4.93		31.81	18.36	< 0.007	17.57	12.54	
IgG	35.00	12.45	< 0.010	35.13	14.30	< 0.020	24.06	7.42	22.73	7.64		38.25	15.10	< 0.004	26.14	13.19	
IgM	38.43	16.54		50.26	29.58		32.93	33.99	36.93	18.12		51.87	21.91	< 0.004	40.19	21.90	
Antigliadin antibodies (Index)																	
IgA	2.73	2.29		7.16	10.85	< 0.030	1.62	0.85	1.85	2.42		14.64	18.15	< 0.005	2.88	2.69	
IgG	2.50	1.64	< 0.006	3.22	2.43	< 0.003	1.30	0.54	1.12	0.90		4.14	3.74	< 0.003	1.18	0.85	
IgM	1.53	0.56	< 0.008	2.00	0.84	< 0.001	1.10	1.80	1.73	0.73	< 0.003	2.15	1.83		1.64	1.38	< 0.010
Anti-DNA antibodies (Index)																	
IgA	1.76	2.09		4.83	7.75	< 0.040	1.23	0.74	1.14	0.65		3.70	4.76	< 0.030	1.88	1.44	
IgG	1.34	1.18		3.60	7.55		1.11	0.94	0.78	0.78		2.16	1.82	< 0.030	1.96	2.56	
IgM	1.82	0.85	< 0.040	1.73	0.79		1.35	0.50	1.46	0.56		1.93	1.03		2.00	0.90	

Target groups are: I, hepatosplenic schistosomiasis, no proteinuria; and II, hepatosplenic schistosomiasis, glomerulopathy. Control groups are: III, normal volunteers; IV, uncomplicated intestinal schistosomiasis; V, non-schistosomal chronic liver disease; VI, primary nephrotic syndrome.

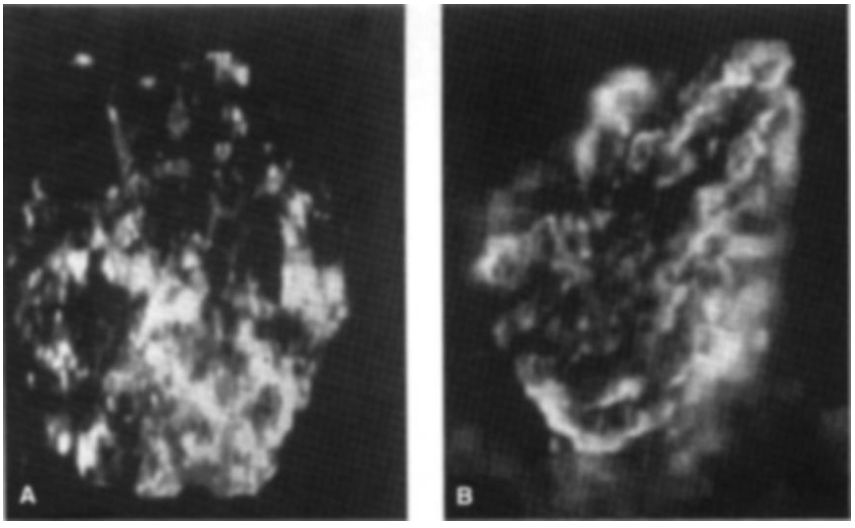

Fig. 1. *Glomerular immunoglobulin deposits in Group II patients, seen by immunofluorescence.* (**a**) IgM mesangial deposits in an early case (Class I) of schistosomal glomerulopathy. (**b**) IgA mesangial and sub-endothelial deposits in an advanced case (Class III).

were also insignificantly less often hypertensive (4.7% compared to 11.7%). Their mean creatinine clearances were matching (means 71.21 ± 24.34 and 73.74 ± 25.00 ml/min, respectively).

As shown in Table 3, there was a polyclonal increase in the total serum immunoglobulin concentrations reaching statistically significant levels for IgG and IgM in Group I, IgA and IgM in Group II, and all three immunoglobulins in Group V. Circulating immune complexes of all immunoglobulin classes addressed were also significantly increased in the mentioned three groups.

In addition, there was a significant selective increase in IgG and IgM anti-gliadin antibodies, IgG rheumatoid factor and IgM anti-DNA antibody activity in Group I. This pattern was carried over, but modified in favor of IgA, to Group II where a 442% increase in the mean IgA anti-gliadin antibodies (compared to normal controls, $P < 0.03$), and a shift of anti-DNA antibody activity to IgA (393% compared to normal controls, $P < 0.04$), at the expense of IgM, were observed (Figs. 1 and 2). IgA predominance was even more pronounced in Group V, where the rise of mean IgA anti-gliadin antibodies reached 904% ($P < 0.005$), and that of IgA anti-DNA activity 301% ($P < 0.03$) at the expense of IgM. A significant increase in rheumatoid factor activity including that of IgA (186%), IgG (159%) and IgM (158%) was also noticed in this group.

The only significant change observed in Group IV was a 157% increase in the mean IgM anti-gliadin antibodies ($P < 0.003$). The changes observed in Group VI were variable, reflecting the diversity of etiology.

There was a statistically significant increase in the frequency of

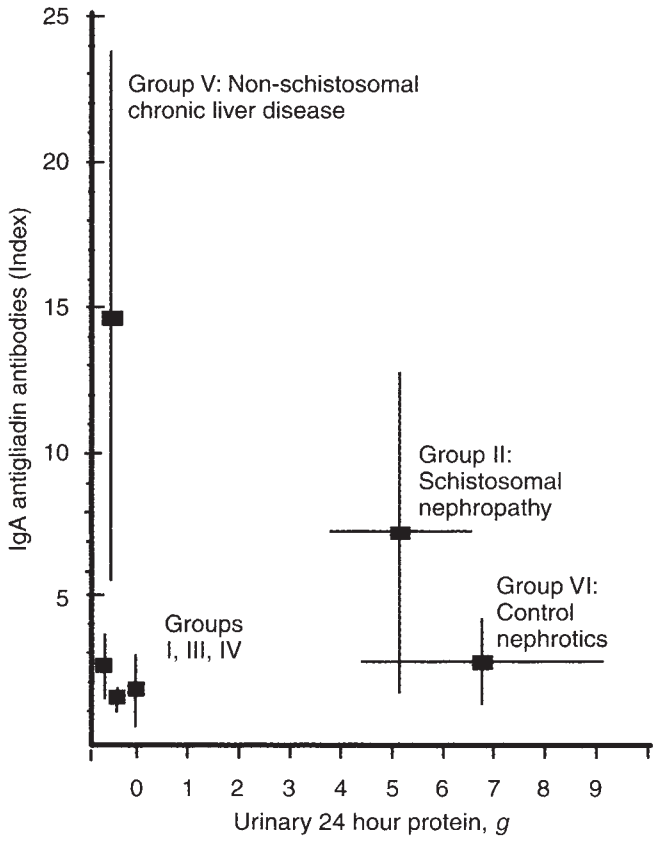

Fig. 2. *Urinary 24-hour proteins in relation to serum anti-gliadin IgA (means and standard errors) in the different groups.*

IgA deposits (Fig. 1b) in Group II cases, compared with control nephrotics (Group VI). Within the former, IgA deposits were encountered, usually along with other immunoglobulins and complement, in 70% of the biopsies showing more advanced lesions (AFRAN [33] Classes III to V) compared to 29% in milder forms of schistosomal glomerulopathy (Classes I and II), (Table 4). The deposits were most often mesangial, but subendothelial and extramembranous deposits were also noticed in 29 and 18% of cases, respectively, again mostly seen in AFRAN Class III cases. In contrast, IgM mesangial deposits were significantly more often encountered in early (Classes I and II) lesions.

Discussion

Three partners interact in the pathogenesis of schistosomal glomerulopathy: the parasite, the liver and the glomerulus. The parasite is a blood fluke that inhabits the portal vein, into which it pours a large load of antigens, mostly originating in its alimentary canal [34]. A number of these antigens have been clearly identified in the glomeruli of experimental animals and patients with schistosomal glomerulopathy [35–37]. Parasitic ova are laid in the colonic submucosa, where they trigger a chronic granulomatous reaction to soluble antigens that diffuse into the surroundings via micropores in the egg shells [38]. The initial phases of the schistosomal granuloma are largely mediated by Th_1 cytokines. The subsequent release of Th_2 cytokines, particularly IL-10 and IL-4 [39–41], is important in checking the intensity of the local reaction and consequently the local pathology. The array of interleukin release in the schistosomal granuloma may have important reflections on the surrounding macrophages and lymphoid aggregations.

The hepatic lesions are basically the same. Granulomata develop around metastatic ova in the portal tracts, ending up with fibrosis and pre-sinusoidal portal hypertension. The hepatocytes are spared from the direct effects of the granulomatous lesions, though they may be involved in the subsequent autoimmune reactions associated with schistosomiasis [42–45]. The hepatic macrophages, on the other hand, are down-regulated by parasitic antigens [43] and late cytokines released in the schistosomal granulomata [40, 41].

The glomerular lesions, in contrast to the mucosal and the hepatic, are attributed to the adult worm antigens [35–37]. Escaping clearance by the inhibited hepatic macrophages [46], the latter are deposited in the mesangium leading to a proliferative response, which has been well documented in many experimental animals [47] and humans [15] infected with any of the major species of human-pathogenic schistosomes. Progression into glomerulosclerosis, however, has been mainly described in *S. mansoni* infection [14, 48], and is usually associated with significant hepatic fibrosis [12, 14, 16, 20, 21, 36].

Based on experimental data in mice [22, 49] and clinical observations in humans [21, 36], IgA was suggested to be a potential mediator in the progression of the glomerular lesions in schistosomiasis. This hypothesis is further examined in the present work.

Two subsets of patients with hepatosplenic schistosomiasis, without and with clinically overt renal involvement (Groups 1 and 2, respectively) constitute the target groups. Since the study parameters are potentially vulnerable to all the major players in the scene, that is, the parasite, the liver and the glomerulus, the independent influence of each of these variables was envisaged through a selected control patient cohort (Groups IV, V and VI, respectively).

The total serum immunoglobulin concentrations in our normal controls (Group III) was a bit higher than expected, which is a fairly common observation among normal Egyptians (Genin, unpublished data), often attributed to their frequent exposure to infections and other antigens in the environment.

Control subjects with chronic non-schistosomal liver disease (Group V) displayed the usual polyclonal immunoglobulin response reported in many previous studies. The IgA predominance noticed in this group also conforms with previous observations, being attributed to its impaired hepatic clearance [50–52] and possibly to increased generation by the gut mucosa [51, 52]. The same mechanisms may explain the remarkable increase in the levels of circulating immune complexes observed in this group. Despite the remarkable elevation of different forms of serum IgA antibodies, no patient in this group showed any clinical evidence of renal disease. Since renal biopsy was not undertaken in this group for ethical reasons, we cannot exclude the occurrence of subclinical lesions. It is notable, though, that lesions reported in similar cohorts are usually mild and have limited clinical sequelae [53, 54].

Control subjects with non-schistosomal nephrotic syndrome (Group VI) had variable immunoglobulin profiles compatible with etiology. It is remarkable, however, that none had any glomerular IgA deposits, which confirms other reports emphasizing the rarity of IgA nephropathy in African populations [1].

Table 4. Glomerular histopathology and immunofluorescence findings in patients with established schistosoal glomerulopathy compared with those with the primary nephrotic syndrome

Case number	Light microscopy	AFRAN class	IgA deposits				IgM deposits	IgG deposits	C3 deposits	Fibrin deposits
			Total	Mesangial	Subendothelial	Subepithelial				
1	Minimal change	Ia	−	−	−	−	+	−	+	−
2	Minimal change	Ia	−	−	−	−	+	+	+	−
6	Minimal change	Ia	−	−	−	−	+	−	+	−
7	Axial mesangioproliferative	Ic	−	−	−	−	+	+	−	−
3	Axial mesangioproliferative	Ic	+	+	−	−	+	−	+	−
15	Axial mesangioproliferative	Ic	+	−	+	−	+	+	+	−
17	Exudative	II	−	−	−	−	+	−	−	+
8	Mesangiocapillary (type I)	IIIa	−	−	−	−	+	−	−	+
5	Mesangiocapillary (type I)	IIIa	−	−	−	−	−	−	+	−
4	Mesangiocapillary (type I)	IIIa	+	−	+	−	−	+	+	−
9	Mesangiocapillary (type I)	IIIa	+	+	+	−	−	+	−	+
11	Mesangiocapillary (type I)	IIIa	+	+	+	−	+	+	+	−
10	Mesangiocapillary (type I) with crescents	IIIa	+	+	−	−	−	+	−	+
13	Mesangiocapillary (type III)	IIIb	+	+	−	+	−	−	−	+
16	Mesangiocapillary (type III)	IIIb	+	−	+	+	−	−	−	−
12	Mesangiocapillary (type III)	IIIb	+	+	−	+	+	+	+	−
14	Amyloid	V	−	−	−	−	+	+	+	+
Overall frequency (N = 17)			9 53%	6 35%	5 29%	3 18%	11 65%	9 53%	10 59%	6 35%
Frequency in Classes I and II (N = 7)			2 29%	1 14%	1 14%	0 0%	7 100%	3 43%	5 71%	1 14%
Frequency in Classes III–V (N = 10)			7 70%	5 50%	4 40%	3 30%	3 30%	5 50%	4 40%	4 40%
Frequency in control primary nephrotics (N = 21)			0 0%	0 0%	0 0%	0 0%	7 33%	6 29%	2 10%	5 24%

The only statistically significant finding in patients with simple intestinal schistosomiasis (Group IV), was the increased serum IgM-antigliadin antibodies. This may reflect the local immune response to food antigens [32, 55], as a consequence of mucosal macrophage inhibition in schistosomiasis [43, 56, 57]. The predominantly IgM response is probably the expression of the known response to many parasitic infestations [58], including schistosomiasis [39, 59].

The same finding was carried over into the later phase of disease evolution, namely hepatosplenic schistosomiasis (Group I). Yet the spectrum of serum reactivity was wider, depicting two additional features known about the disease, namely autoimmunity [42–45] and hepatic fibrosis [60, 61]. The former was expressed in this group by a significant increase of serum IgG rheumatoid factor and IgM-anti-DNA activity, while the latter was displayed by a polyclonal increase in serum total immunoglobulins and a significant increase of circulating immune complexes [46, 60, 61].

Switching from the parasite-associated IgM predominance to the liver-geared IgA sovereignty distinguished patients with overt glomerular disease (Group II) from those without (Group I). The former had a statistically significant multitudinous increase in IgA anti-gliadin and anti-DNA antibodies. IgA glomerular deposits were noticed in as many as 53% of cases, compared to none in the control primary nephrotics. Unfortunately, it is impossible to obtain a respective figure for comparison with Group I patients, from whom renal biopsies could not obtained for ethical reasons. However, on the basis of previous observations by some of us [21, 62], such patients are very unlikely to have any IgA deposits. Furthermore, it was noticed in the present study that even within Group II patients, IgA deposits were far less often associated with mild glomerular lesions (29% with Classes I and II compared with 70% in Classes III to V). These figures are higher than those reported in other series [63]; the discrepancy is presumably attributed to variance in the prevalence and severity of associated hepatic fibrosis in different cohorts.

According to these data and others on primary IgA nephropathy [64], it seems obvious that significant IgA glomerular pathogenicity requires a co-morbidity factor that is provided, in our case, by concomitant exposure to schistosomal antigens. It is understandable that the initial parasite-associated proliferative lesions may be associated with critical mesangial modulation necessary for IgA binding and/or pathogenicity [65–67].

On the basis of the present findings, we can only speculate about the mechanisms involved in switching of some patients with hepatosplenic schistosomiasis to the IgA-predominant immunoglobulin profile, and hence developing serious renal disease (Fig. 3). Previous studies had suggested that critical impairment of hepatic clearance may be an important factor [21], and yet this alone does not explain the disproportionate increase in the anti-gliadin and anti-DNA fractions. The significant increase in serum anti-gliadin IgA is suggestive of augmented mucosal generation, as described in celiac disease, which is also characterized by chronic gut mucosal inflammation [32]. Switching of the predominant anti-DNA activity from IgM to IgA may point to a corresponding B-lymphocyte switching analogous to that suggested in primary IgA nephropathy [68–71]. Although we have no cellular data to support this concept, we must admit that the mucosal scene in hepatosplenic schistosomiasis is optimally set for switching: a persistent antigen load [32], macrophage inhibition [66] and Th_2 cytokine predominance particularly involving IL-10 [72, 73]. Committed mucosal immunoblasts could then migrate

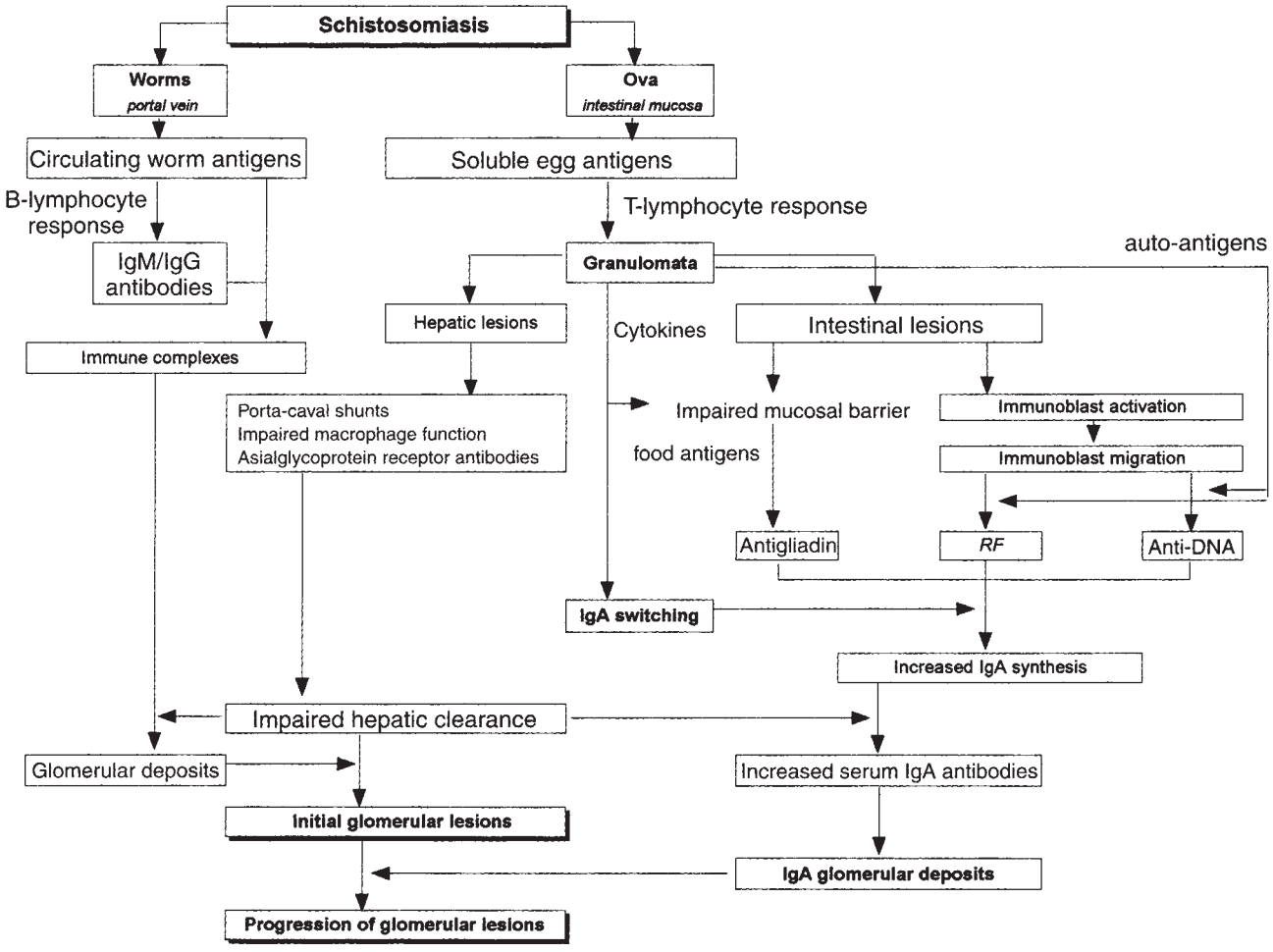

Fig. 3. *Suggested pathogenetic mechanisms in schistosomal glomerulopathy showing the possible immunological interaction between worms in the portal blood and egg-granulomata in the liver and colonic submucosa.* The potential role of IgA within this context is highlighted.

along with the augmented lymphatic flow, known for chronic liver disease [74] and particularly for hepatosplenic schistosomiasis [75], into distant locations, thereby exerting a systemic effect.

It is unclear whether the prominent serum autoreactivity observed in patients with hepatosplenic schistosomiasis has an independent pathogenetic role in the development or evolution of the glomerular lesions. It also remains to be elucidated whether such reactivity contributes to impairment of hepatocyte-dependent IgA clearance via asialoglycoprotein receptors [76], as described in other immune-mediated chronic liver disease [77, 78].

To conclude, this work substantiates the importance of hepatic dysfunction in the progression of schistosomal glomerulopathy. The critical difference between patients with hepatosplenic schistosomiasis who develop overt nephropathy and those who do not is the acquisition of the IgA-dominated "hepatic' immunoglobulin profile. The initial parasite-induced lesions seem to induce the glomerular mesangium for subsequent IgA-mediated progression. Parasite-triggered auto-immune mechanisms may be co-pathogenetic.

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