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Autoantibodies to intermediate filaments in sera of patients with *Schistosoma mansoni* infection

M. W. J. BOEHME, P. K. KATAAHA* & E. J. HOLBOROW* *London School of Hygiene and Tropical Medicine and *London Hospital Medical College, London, England*

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SUMMARY

Autoantibodies to the intermediate filament proteins vimentin and keratin were studied in sera of 50 Caribbean patients with *Schistosoma mansoni* infection and 50 control subjects. Autoantibodies were detected by indirect immunofluorescence on HEp-2 cells pretreated with colchicine. The incidence of anti-vimentin antibodies in patients' sera was 94% for IgM, 12% for IgG, and 4% for IgA; in the control subjects incidence was 52%, 0%, and 4%, respectively. Anti-keratin antibodies were found in 82%, 4%, and 4% of patients' sera and 42%, 0%, and 2% in controls, respectively. The difference between the geometric means of titres for patients (1:150) and controls (1:26) was highly significant ($P < 0.001$). The possible role and genesis of autoantibodies to intermediate filaments is discussed.

Keywords *Schistosoma mansoni* infection autoantibodies vimentin keratin

INTRODUCTION

Schistosomiasis is a world-wide disease affecting more than 300 million people (Capron *et al.*, 1982). The immunology is complex, involving parasitic, species-specific, and host factors as well as humoral and cell-mediated immunity (reviews in Warren, 1972; Smithers & Terry, 1976; Lichtenberg, 1977). Considerable information is available about parasitic escape mechanisms (Dean, 1974; Goldring *et al.*, 1976; Caulfield & Cianci, 1985), concomitant immunity (Smithers, Terry & Hockley, 1969), antibody-dependent, cell-mediated cytotoxicity (Butterworth *et al.*, 1975; Capron & Capron, 1980), and immunomodulation in chronic disease with changes in T cell subsets (Colley *et al.*, 1983; Gastl *et al.*, 1984; Feldmeier *et al.*, 1985), but little is known about the presence and specificity of autoantibodies in sera of patients. Autoantibodies to intermediate filaments (IF) of the cytoskeleton have been observed in a variety of viral, bacterial, protozoal and connective tissue diseases (summarizing table in Senecal, Oliver & Rothfield, 1985). The aim of the present study was to investigate the incidence of these autoantibodies in schistosomiasis.

MATERIALS AND METHODS

Patients and controls

We studied sera from 50 patients from the Caribbean (St Lucia) with *Schistosoma mansoni* infection. The diagnosis was confirmed clinically, immunologically (Schistosoma egg antigens (SEA)-antibody titre), and parasitologically (Table 1). As controls we used 50 sera from patients in Trinidad who had been successfully treated for filariasis with diethylcarbamacepine 1 year earlier with no relapse of disease, and in whom schistosomal egg counts were negative. In addition, we tested 20 sera of healthy laboratory staff without a history of parasite infection.

Correspondence: Dr M. W. J. Boehme, Department of Internal Medicine, University of Ulm, D-7900 Ulm, West Germany.

Screening for autoantibodies

ELISA. The titres for antibodies to SEA and schistosoma worm antigens (SWA) were determined by micro-ELISA technique (Engvall & Perlmann, 1972; modified by Voller *et al.*, 1974). The methods for the preparation of SEA and SWA are described elsewhere (Murare *et al.*, 1987). Plates were coated with either 2 μg/ml SEA (150 μl/well) or 2·5 μg/ml SWA (150 μl/well) as antigens.

The sera were added in a dilution of 1:300 and the conjugates (peroxidase-conjugated, rabbit anti-human immunglobulins; Dako-Immunoglobulins, Denmark) in a dilution of 1:4000 (IgG) or 1:2000 (IgM). The enzymatic reaction using OPD (ortho-phenylene-diamine, BDH Chem., Poole, UK) was measured at 495 nm and calibrated with respect to a reference positive control sample (Titertek Multiskan).

Indirect immunofluorescence assay. HEp-2 cells (human epidermal laryngeal carcinoma) were cultured as a mono-layer on multi-spot slides and treated with colchicine (0·25 μg/ml) 12 h before use. The cell monolayers were fixed in methanol for 10 min at −20°C. All sera were tested at a starting dilution of 1:10. Positive sera were then diluted further up to a titre of 1:320. The conjugate used was fluorescein-labelled sheep anti-

Table 1. Characteristics of 50 *S. mansoni*-infected patients and 50 control subjects

	Patients			Controls		
	M		F	M		F
n	25		25	33		17
Age (years)						
mean	19·4±1·7		28·8±3·6	42·7±3·7		31·2±4·5
Range	6–41		7–77	8–79		10–60
Faecal smear for	+	++	+++	+	++	+++
S. mansoni eggs	46%	18%	36%	0%	0%	0%
Quantitative egg count						
mean	129·5±28·3			—		
Range	11–1117			—		

Egg extraction was measured as number of ova/1g of stool.

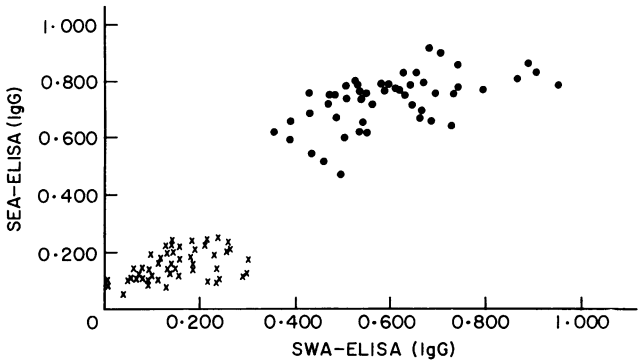

Fig. 1. Correlation between absorbance values (at 492 nm) for the SEA- and SWA-ELISA in sera of patients with schistosomiasis (●) and controls (x).

human IgG, IgM, or IgA (Wellcome Reagents, UK). The stained preparations were mounted in 90% glycerol containing 25 gm/*l* diazobicyclo-octane (Sigma, St Louis, MO) to prevent fading (Johnson *et al.*, 1982). The slides were viewed under a Zeiss microscope (objective: Planapo 40; 0·95) with the filter combination ref.L.P. 510 nm; K.P. 490 nm; K.P. 500; Barr L.P. 520 nm.

Statistical analysis

The data were compared using a double-tailed Student's *t*-test. Linear and logarithmic regression analysis were also performed.

RESULTS

In both ELISA-tests used (SEA and SWA) there was a significant difference in disease-specific antibodies between the patients with *Schistosoma mansoni* infection and the control samples (Fig. 1). The differences were significant for both SEA and SWA ($P < 0{\cdot}001$).

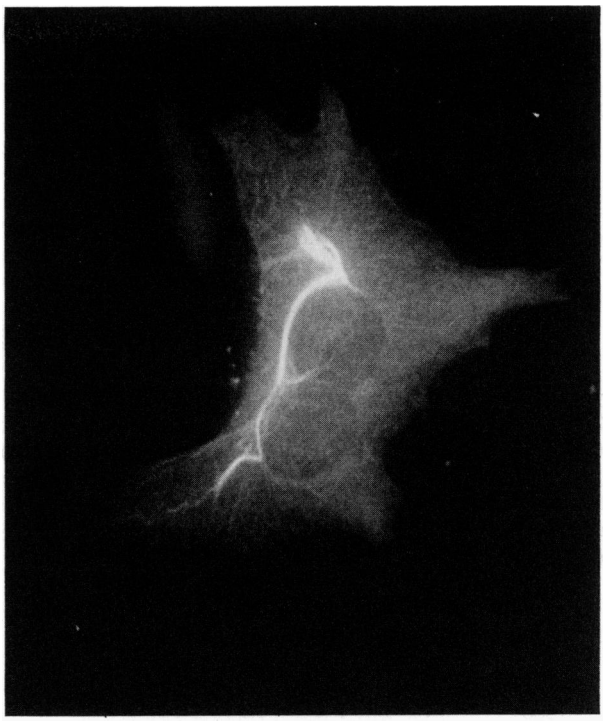

Fig. 2. HEp-2 cell treated with colchicine and stained with a serum of a patient with *Schistosoma mansoni* infection (IgM). Note the characteristic staining pattern of coiled vimentin filaments.

Table 2. Incidence of autoantibodies to IF in patients with *Schistosoma mansoni* infection and controls

Autoantibodies to IF	Sera (*n*)	Vimentin staining pattern (% positive)			Keratin staining pattern (% positive)		
		IgM	IgG	IgA	IgM	IgG	IgA
Patients	50	94	12	4	82	4	4
Control	50	52	0	4	42	0	2

It is possible to distinguish between staining for the IF proteins vimentin and keratin on HEp-2 cells. On pretreatment with colchicine, the vimentin filament system collapses around the nucleus and is distinguishable from the fine keratin network by its resultant coiling (Fig. 2).

Most of the autoantibodies to IF found belonged to the IgM-class. Not only was the incidence of autoantibodies to the IF proteins vimentin and keratin higher in patients with *Schistosoma mansoni* infection compared with controls (Table 2), but their titre was much higher in the positive samples. This is shown in Fig. 3 for autoantibodies to vimentin, the final titre of which is more precisely determinable because of the clarity of the coiled staining pattern. Control sera from laboratory staff revealed no positive staining patterns.

Any correlation between the titre of disease-specific antibodies (anti-SEA or -SWA antibodies) and of anti-IF autoanti-

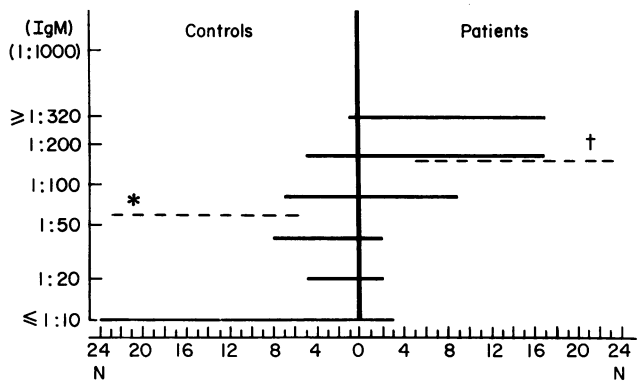

Fig. 3. IgM-specific anti-vimentin autoantibody titres of sera from 50 patients with schistosomiasis and 50 control subjects, as determined by indirect immunofluorescence on HEp-2 cells. The horizontal lines show the number of positive samples. Geometric means for positive samples. (>titre1:10) * controls: $1:60\pm1$; and † patients: $1:156\pm1$ ($P<0.001$).

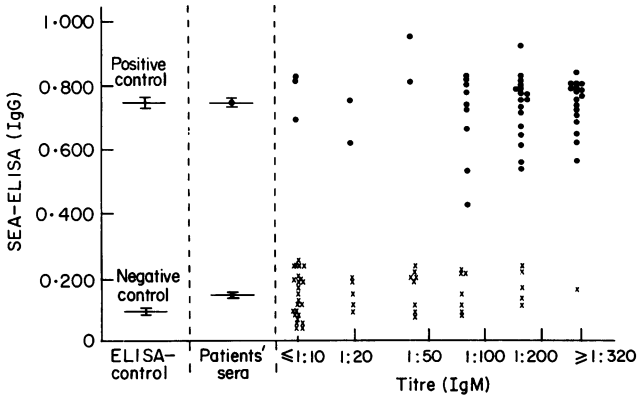

Fig. 4. Correlation between absorbance values (at 492 nm) of anti-SEA antibodies of IgG class (a serological disease marker) and titres of anti-vimentin antibodies of IgM class (the most common class of autoantibodies to IF) in patients with schistosomiasis (●) and controls (x). Correlation was not detected. The means of the ELISA-control values and of the serum samples are shown.

bodies was also looked for. Figure 4 shows no significant correlations between levels of SEA antibodies (IgG) and the autoantibodies to IF (IgM). Nevertheless, a tendency was observed for the titre of autoantibodies to IF to correlate with the egg output, that was age dependent. The egg output was higher in women than in men and had its highest value in the age group 10–20 years. This age group also had the highest titres of autoantibodies to IF (data not shown).

DISCUSSION

In patients with *Schistosoma mansoni* infection autoantibodies have been described to normal liver and lung tissue (Shamma, Ali & El-Shawi, 1965; Bassily *et al.*, 1973), sperm (Abdal *et al.*, 1975), DNA (Hillyer, 1971; Carlier *et al.*, 1976), antinuclear antibodies (Bendixen *et al.*, 1984; Pun *et al.*, 1984) and globulin-rheumatoid factor (Abdin *et al.*, 1970; Lehman *et al.*, 1972; Carvalho *et al.*, 1983).

In this study, autoantibodies to IF were observed in schistosomiasis not only with higher incidence than in controls but also with a higher titre. Their specificity was demonstrated as against vimentin and keratin by immunofluorescent staining pattern on HEp-2 cells pretreated with colchicine, which depolymerizes micro-tubules (Ishikawa, Bischoff & Holtzer, 1968). It is well known that autoantibodies to cytoskeletal components occur in control samples also, but less frequently and in lower titre (Guilbert Dighiero & Avrameas, 1982, Mortazavi-Milani, Badakere & Holborow, 1984). Moreover, the titres of these autoantibodies are frequently related to disease activity (Bretherton, Toh & Jack 1981; Mortazavi-Milani, Facer & Holborow, 1984).

It is interesting to note that a relation appears to exist between autoantibody titre to IF and severity of schistosoma infection (egg count). Therefore, tissue damage due to penetration of eggs may trigger the production of anti-IF-antibodies. It has been shown that anti-IF autoantibodies can bind to the cytoskeleton of injured cells and they may thus be involved in the removal of such cells (Hansson & Schwartz 1983; Hansson *et al.*, 1984), especially as anti-IF-antibodies are known to activate complement (Linder, Letho & Stenman, 1979). One explanation of their production may be polyclonal B-cell activation due to parasite-derived products. This has been shown already for malarial-derived products (Kataaha *et al.*, 1984), leishmaniasis (Boehme *et al.* 1986), and infection with the Epstein–Barr virus (Mortazavi-Milani, Stierle & Holborow, 1982). It seems possible that SEA can induce this response due to its capacity to trigger lymphocyte blastogenesis (Colley, 1972; and authors' experiments, data not shown). Alternatively, cross-reactivity (molecular mimicry) with antoantibodies and IF antibodies has been documented for several viruses (Fujinami *et al.*, 1983; Oldstone, 1987), but the absence of correlation between SEA/SWA (using crude egg or worm extracts) and IF-antibody levels reduces the likelihood of this kind of cross-reactivity in schistosomiasis.

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