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## **Immunodiagnosis of schistosomiasis outside endemic areas: use of histamine release from basophils by schistosomal antigens and radioallergosorbent test**

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### **Summary**

Specific allergic histamine release from leucocytes, radioallergosorbent tests (RAST) and indirect fluorescent antibody tests (IFAT) were applied for immunodiagnosis of schistosomiasis in patients outside endemic area. Of 10 parasitologically verified cases – all of them exhibiting a low and irregular egg output – 8 were detected by histamine release, whereas 4 patients with filariasis, 3 with trichuriasis and 31 parasitologically normal controls were negative in this respect. By a combination of the histamine release test, RAST and IFAT all 10 cases were diagnosed. RAST and IFAT applied to 29 patients with active or treated schistosomiasis were positive in 14 (48%) and 20 (69%) cases, respectively.

*Key words:* Schistosomiasis; immunodiagnosis; histamine release; IgE antibodies; effects of antischistosomal treatment.

### **Introduction**

Schistosomiasis in patients in non-endemic areas is frequently characterized by a small worm burden, a low and irregular egg output and inconspicuous clinical symptomatology and may, therefore, give rise to diagnostic difficulties. In an attempt to cope with these difficulties we have been evaluating various immunological test systems. In a recent publication we assessed the potential usefulness of the radioallergosorbent test (RAST) for the serodiagnosis of schistosomiasis and compared it with the indirect fluorescent antibody test

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(IFAT) (Weiss et al., 1978). We found that in African patients repeatedly exposed to cercarial infestation the RAST for specific IgE antibodies to *S. haematobium* and *S. mansoni* compares favourably with the IFAT in specificity and sensitivity.

In the present study, we have extended the above examinations to include irregularly exposed patients returning from endemic areas to Europe. In addition, we report results on specific allergic histamine release (HR) from basophils, a novel method in the immunodiagnosis of schistosomiasis.

## Patients

Two groups were studied. The patients in group A (Table 1) were with one exception (J. B.) Europeans. Schistosomiasis was parasitologically confirmed in 11 cases within 3 to 12 months of their returning to Switzerland. In all these cases the HR tests was performed, as well as the RAST and the IFAT. Histamine liberation from leucocytes from patient G. E. was not assessed until 12 months after the initiation of treatment (see Table 3).

Group B (Table 4) consisted exclusively of Europeans who had been staying in endemic areas for periods ranging from about one year to as long as 27 years. The 20 subjects listed as controls had returned home after a stay of several months to a few years duration in countries where schistosomiasis is endemic and were found to be free of parasites.

The patients in both groups were examined at the Medical Department of the Swiss Tropical Institute. They reported either for routine examination or because of symptoms suggesting schistosomiasis or other parasitic diseases. All their relevant personal particulars, especially with regards to history of exposure, were obtained by means of a standard questionnaire (Stürchler, 1979).

## Methods

*Parasitological techniques.* Urine was either centrifuged or filtered (millipore filter) for eggs of *S. haematobium*. Faeces were examined by standard techniques for eggs of *S. mansoni* and *S. intercalatum* (Garcia and Ash, 1979). In cases that were parasitologically negative, but had a history of exposure and symptoms suggestive of schistosomiasis, with positive immunological findings or with blood eosinophilia not explained by other causes, rectoscopy was performed and a routine biopsy of the rectal mucosa obtained. The mucosa specimen was soaked in Faures solution (Geigy and Herbig, 1955) for 24 h to render it translucent, squeezed between two slides and then examined microscopically for eggs of *S. haematobium* and *S. mansoni* (Pieron et al., 1980).

*Immunological procedures.* The preparation of crude schistosomal antigens, the radioimmunosorbent test for total serum IgE, the RAST for antischistosomal IgE and the IFAT were carried out as described previously (Weiss et al., 1978). Only RAST classes 3 and 4 were considered positive, whereas the cut-off point in the IFAT was set at a serum dilution of 1:160.

*Release of histamine from basophils by schistosomal antigens.* Leucocytes obtained by venipuncture were prepared according to May et al. (1970), washed in Tris-buffer pH 7.7 containing albumin, calcium and magnesium (Tris ACM) (May et al., 1970) and resuspended in this medium at a concentration of  $2-5 \times 10^7$  cells/ml. Antigen in various concentrations or medium alone was added to the cells, and the mixture shaken for 1 min and incubated for 15 to 30 min at 37°C. The samples were then centrifuged (375 g, 5 min, 4°C) and the supernatants processed for histamine determination. Histamine was quantitated fluorometrically (Shore et al., 1959; von Redlich and Glick, 1965) or by means of a modified (Zingel and Dietrich, in preparation) enzymatic isotopic assay (Beaven et al., 1972; Levy and Widra, 1973; Shaff and Beaven, 1979). In either case, automated procedures were used for extractions, further processing, measuring and recording (Siraganian, 1976). In the case of the fluorometric determination, incubation mixtures consisted of 250 µl of cells

and an equal amount of antigen. After incubation, the cells were removed by centrifugation, and the supernatants were deproteinized with 0.4 N perchloric acid before proceeding to the sample collector. For the enzymatic isotopic assay of histamine, cells and antigen (20  $\mu$ l each) were pipetted into microtitre plates, shaken, incubated and centrifuged. To 20  $\mu$ l of supernatants, transferred to microtitre plates, 10  $\mu$ l of S-(methyl- $^{14}$ C)adenosylmethionine was added in the presence of histamine N-methyl transferase. The plates were incubated for 2 h at 37° C. Perchloric acid was added, and after centrifugation the supernatants proceeded to the sample collector.

The total histamine content of the cells was determined after perchloric acid treatment (fluorometric assay) or heating for 5 min in a boiling water bath (enzymatic isotopic assay). Spontaneous release was assessed in the supernatants of samples incubated in the absence of antigen. Histamine release was calculated according the following formula and expressed as a percentage:

$$\text{HR} = \frac{\text{sample histamine} - \text{spontaneous release}}{\text{total histamine} - \text{spontaneous release}} \times 100$$

Spontaneous release averaged  $4.2 \pm 2.2\%$ , with a range from 0 to 10 (n = 49), of the total histamine content. Calculations involved in histamine quantitation, the construction of standard curves and the readings therefrom were performed with the aid of a computer programme devised and kindly provided by Dr. H. Huber, Ciba-Geigy Ltd.

## Results

Data on histamine liberation from leucocytes by schistosomal antigens are shown in Table 2 and Fig. 1. Significant amounts of histamine were released in 8 of the 10 patients suffering from schistosomiasis, whereas in the 7 patients with

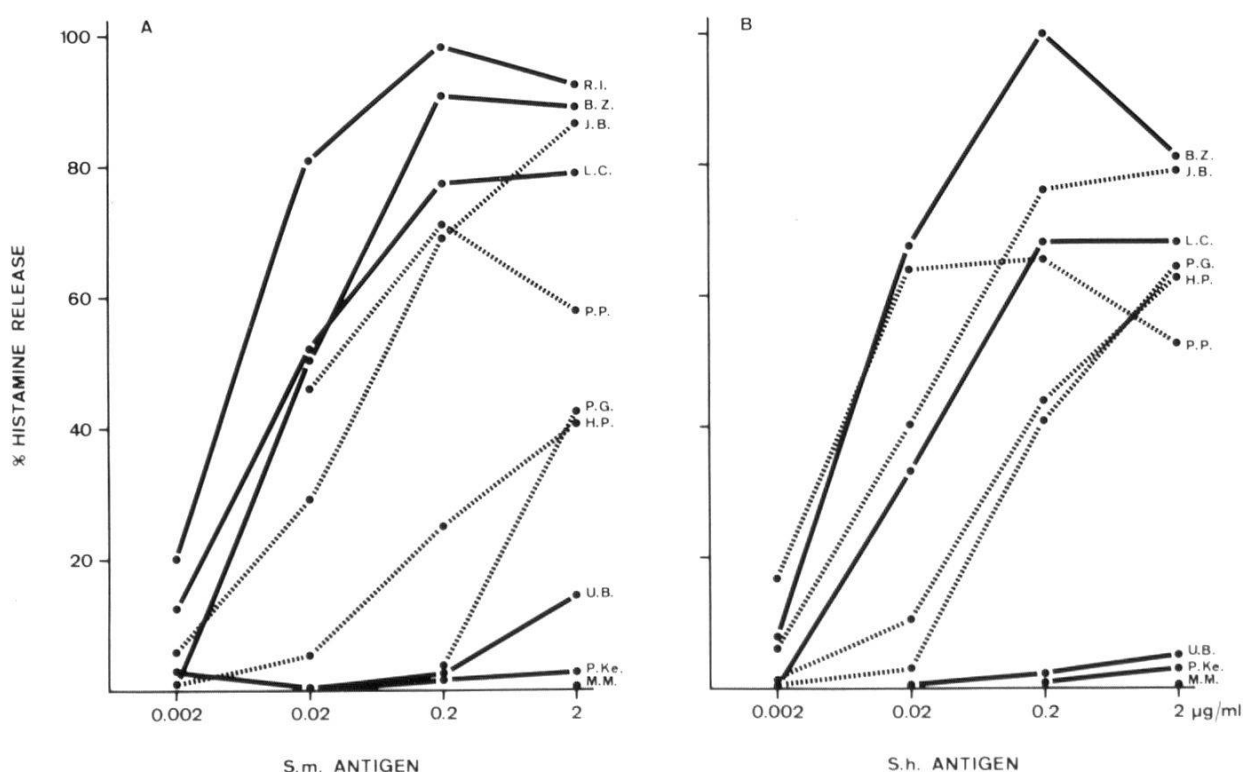


Fig. 1. Histamine release by schistosomal antigens from blood leucocytes of patients with parasitologically verified schistosomiasis. Histamine release by *S. mansoni* antigen (panel A) and *S. haematobium* antigen (panel B). Solid lines: *S. mansoni* infections; dotted lines: *S. haematobium* infections.

Table 1. Study group A: clinical and parasitological characteristics

Initials	Sex	Age	Years in endemic areas	Examination for	Leucocytes ( $\times 10^9/l$ )	Eosinophils (%)
<i>S. mansoni</i>						
B. Z. ....	♂	62	23	routine	8.6	14.0
U. B. ....	♂	31	2	routine	5.9	6.0
L. C.* ....	♂	38	2	routine	5.8	14.0
M. M.* ....	♂	26	2	routine	8.7	5.0
P. Ke. ....	♂	42	1	eosinophilia	5.5	37.0
R. I. ....	♂	36	12	routine	6.0	5.5
G. E. ....	♂	31	20	urticaria	6.1	12.5
<i>S. haematobium</i>						
P. P. ....	♂	36	6	haematuria	10.5	12.0
H. P. ....	♂	54	25	haematuria	4.5	5.5
P. G.* ....	♂	31	5	pruritus	9.9	26.5
J. B. ....	♂	14	14	haematuria	4.5	22.5
<i>Onchocerca volvulus</i>						
H. E. ....	♀	50	18	pruritus	5.3	33.5
E. W. ....	♀	36	3	pruritus	4.4	4.0
<i>Loa loa</i>						
N. T. ....	♂	47	20	migratory oedema	10.8	19.0
M. D. ....	♂	24	4	migratory oedema	7.4	19.0
<i>Trichuris trichiura</i>						
P. Kl. ....	♂	39	7	routine	7.5	2.5
J. R. ....	♂	35	12	routine	4.1	1.0
P. L. ....	♂	30	1	routine	8.9	0.5

\* Concomitant parasitic infections: L. C.: *Entamoeba histolytica*, M. M.: *Ascaris lumbricoides*, P. G.: *Schistosoma intercalatum*

onchocerciasis, loiasis or trichuriasis no histamine liberation was detected. For control of the method, leucocytes from 31 healthy Swiss residents were tested and gave negative results. Histamine release in this group averaged  $0.6 \pm 1.2\%$ , with a range from 1 to 5.9%. As noted previously (Weiss et al., 1978), there seems to be a complete immunological cross-reactivity between the *S. mansoni* and *S. haematobium* systems (Fig. 1). However, in the homologous system the antigen concentration required for significant release was somewhat less than in the heterologous system. A delipidized antigen preparation from adult *S. haematobium* worms and saline-extracted crude antigens gave similar results.

Among the schistosomiasis patients, all but 2 (Table 2) had increased total serum IgE. The concentration did not correlate with the length of stay in the endemic area as indicated in Table 1. 5 of 10 schistosomiasis patients reacted in classes 3 and 4 in the RAST and also patient G. E. after specific treatment (Tables 2 and 3). An additional 2 patients reacted in class 2. Six of 7 controls

Table 2. Study group A: immunological findings

	Maximum histamine release (%)			Total serum IgE (U/ml)	Antibodies to schistosomes		
	antigen				RAST	IFAT	
						antigen	
	<i>S. m</i>	<i>S. h.</i>	<i>S. h. *</i>		<i>S. m.</i>	<i>S. h.</i>	<i>S. m.</i>
<i>S. mansoni</i>							
B. Z. ....	91	100	91	3050	4**	4**	320***
U. B. ....	15	5	11	63	0	0	160
L. C. ....	79	68	76	1100	3	3	80
M. M. ....	0	0	0	640	0	0	320
P. Ke. ....	0	0	0	450	3	3	480
R. I. ....	98	ND	ND	980	2	2	160
<i>S. haematobium</i>							
P. P. ....	71	66	71	4750	4	4	<80
H. P. ....	41	64	ND	185	0	ND	<80
P. G. ....	42	64	ND	750	2	ND	160
J. B. ....	86	79	78	5250	3	4	80
<i>Filariids</i>							
H. E. ....	3	2	5	1200	0	0	<80
E. W. ....	5	1	ND	105	0	ND	<80
N. T. ....	3	3	ND	10000	2	2	<80
M. D. ....	1	ND	0	50	0	ND	<80
<i>T. trichiura</i>							
P. Kl. ....	0	ND	0	130	0	ND	<80
J. R. ....	1	ND	0	145	1	ND	<80
P. L. ....	1	ND	0	10	0	ND	<80

\* delipidized antigen

\*\* class

\*\*\* reciprocal dilution

ND = not done

reacted in classes 0 or 1, and only 1 individual, a patient with loiasis and high total IgE, in class 2. Finally, IFAT was positive in only 6 schistosomiasis cases and negative in all the controls.

As shown in Table 3, various immune parameters were assessed in three patients before and after chemotherapy. Maximum histamine release and the RAST levels did not change. Total serum IgE, on the other hand, was somewhat lower, whereas IFAT titres tended to be higher, after completion of treatment.

In Group B (Table 4), the serological results were compared with the parasitological findings. RASTs and IFATs were positive in 7 and 8 cases, respectively, out of 13 parasitologically verified cases of schistosomiasis. In a group consisting of 21 subjects either treated for schistosomiasis or having a history of exposure, 9 had a positive RAST and 16 a positive IFAT. In 28 controls no positive RAST was seen, the IFAT was positive in 7 cases.

Table 3. Immunological findings obtained before and after initiation of chemotherapy<sup>Δ</sup>

	Months after treatment	Maximum histamine release (%)			Total serum IgE (U/ml)	Antibodies to schistosomes		
		antigen				RAST	IFAT	
						antigen		
		<i>S. m.</i>	<i>S. h.</i>	<i>S. h.*</i>		<i>S. m.</i>	<i>S. h.</i>	<i>S. m.</i>
<i>S. mansoni</i>								
U. B. ....	0	15	5	11	63	0**	0	160***
	10	10	10	7	48	0	ND	< 80
G. E. ....	0	ND	ND	ND	4000	4	3	320
	0.5	ND	ND	ND	3000	4	3	480
	1	ND	ND	ND	10750	3	3	1440
	12	88	91	88	950	4	ND	480
<i>S. haematobium</i>								
J. B. ....	0	86	79	78	5250	3	4	80
	2	68	63	69	3850	3	ND	320

<sup>Δ</sup> U. B. and G. E. were treated with oxamniquine (Vansil), total dose 60 mg/kg, J. B. with niridazole (Ambilhar), total dose 150 mg/kg

\* delipidized antigen

\*\* class

\*\*\* reciprocal dilution

ND = not done

## Discussion

Ten patients with parasitologically verified schistosomiasis were investigated for histamine release (HR) before, and 3 after specific antischistosomal treatment. In all of them signs and symptoms of schistosomiasis were slight. Only one patient (M. M.) presented with a concomitant helminthic infestation.

HR turned out to be a sensitive immunological test. A significant HR, i. e. 15% or more of the total histamine concentration, could be demonstrated in 8 of 10 patients before treatment and in an additional patient after it. Among those with positive HR two separate groups could be distinguished: "high responders" comprising 5 patients who released histamine on stimulation with low concentrations of schistosomal antigen, ranging between 0.002 to 0.02 g/μl (Fig. 1), as opposed to 3 "low responders" who needed an antigen concentration 10 to 100 times higher for significant HR. When crude saline extracts from adult worms are used, *S. mansoni* and *S. haematobium* show essentially a total immunological cross-reactivity. These extracts, however, may not be adequate for recognizing the stages of infestation. Recent investigations by Lunde et al. (1979) have shown that the immune response of the host is influenced by the stage of infestation and by the intensity of exposure. This may explain the



Table 4. Study group B: immunological findings

	Number of sera tested	Total serum IgE (U/ml)	Number with antibodies to schistosomes						
			RAST <sup>Δ</sup>			IFAT <sup>Δ</sup>			
			0/1	2	3	4	<80 <sup>ΔΔ</sup>	80	≥160
<i>Schistosomiasis</i>									
active*	13	1030 (50–5975)□□	3	3	4	3	2	3	8
treated**	14	360 (30–10800)	4	3	4	3		2	12
history of exposure**	7	245 (10–860)	4	1	2			3	4
Other helminthiasis□	8	240 (10–10000)	7	1			7		1
Controls	20	50 (<10–275)	20				14		6

\* parasitologically positive

\*\* parasitologically negative

□ *Onchocerca volvulus* (2), *Loa loa* (2), *T. trichiura* (4)

□□ geometric mean (range)

Δ class; antigen *S. m.*

ΔΔ reciprocal dilution



negative HR test in patients P. Ke. and M. M. who did not present with symptoms related to schistosomiasis and had stayed for only 1 and 2 years in endemic areas.

HR was specific for schistosomiasis patients and none of the patients with filarial infestations or with trichuriasis released significant amounts of histamine (Table 2). In this regard patient N. T. with a high concentration of total IgE (10000 U/ml) is most remarkable. HR indicates the presence of specific IgE antibodies bound to cells, as originally described by Lichtenstein and Osler (1964), while RAST detects the freely circulating, "excess" portion of specific IgE. It is therefore interesting to compare the results of HR and RAST in our patients (Table 2). "High responders" achieved higher RAST classes (class 2 or above) than did "low responders", of whom 2 were in class 0. This seems to suggest that when there are few specific IgE antibodies available RAST is a less sensitive test than HR. This view is supported by the results obtained in patients H. P. and U. B. in whom the concentration of total IgE was low, and concurrently their RAST classes 0, while they still had sensitized leucocytes as indicated by a positive HR.

In our previous work we demonstrated that among 136 African patients with schistosomiasis more than 80% could be correctly diagnosed by means of the RAST (Weiss et al., 1978). In that autochthonous population the geometric mean concentration of total IgE was 3450 U/ml, and only RAST classes 3 to 4 were considered positive. In the present study mostly patients returning to Europe with low-grade infestation and with mean concentration of total IgE of 1030 U/ml were investigated. If only RAST classes 3 to 4 are considered positive, the RAST was less sensitive (7 of 13 patients, 54%).

The medical and parasitological follow-up within 12 months after specific treatment showed that all patients had been cured. Though it is very likely that in patients U. B. and G.E. (Table 3) adult worms were completely destroyed, eggs calcified and the miracidia no longer excreting antigens 10 and 12 months after treatment, the leucocytes remained sensitized and continued to release histamine after stimulation. However, the total IgE concentration in patient G.E. had decreased about 5-fold by that time. The titres of IgG and IgM specific antibodies, as measured by IFAT, showed a rise 1 to 12 months after treatment, as described by Ambroise-Thomas (1969).

Because of the rather sophisticated technique needed for measuring histamine concentrations, it is unlikely that HR will be included in the near future among the various test systems used for the routine diagnosis of schistosomiasis (Sadun, 1976; McLaren et al., 1978; Hillyer et al., 1979; Lunde et al., 1979), although a simplification has recently been proposed using  $^3\text{H}$ -histamine incorporation (Stahl Skow et al., 1979).

However, studies of HR may shed light on mechanisms of sensitization in patients with schistosomiasis, particularly in the increasing number of people returning from endemic areas with this infestation (Gsell, 1978; Laverdant et

al., 1980; Pieron et al., 1980). Similar studies in patients with schistosomiasis (Catto et al., 1980) and tropical eosinophilia have been published while our investigations were in progress (Ottesen et al., 1979).

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