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## Liver involvement in human schistosomiasis mansoni

### Regression of immunological and biochemical disease markers after specific treatment<sup>3</sup>

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### Summary

Peripheral blood cholyglycine and procollagen-III-peptide were measured in 22 Zairean patients with hepatomegaly caused by *S. mansoni* before and after treatment with praziquantel. Circulating T-cell subsets and cutaneous in vivo delayed type hypersensitivity were assessed; serum neopterin and  $\beta_2$ -microglobulin served as indicators for macrophage/lymphocyte activation. The results were compared to age and sex matched patients with *S. mansoni* infection limited to the intestinal tract and schistosomiasis free controls with equal socioeconomic background. Abnormal serum cholyglycine and neopterin levels and alterations of circulating T-cell subset frequencies were associated with hepatomegaly in schistosomiasis. Normalization of these parameters reflected a regression of egg-induced immunopathology as early as two months after specific chemotherapy. Serum procollagen-III-peptide concentrations rose significantly after treatment, suggesting release of propeptide previously incorporated without cleavage into tissue collagen. The combination of these biochemical and immunological parameters may allow assessment of the pathophysiological mechanisms responsible for liver disease in individual patients.

**Key words:** *Schistosoma mansoni*; liver fibrosis; cell-mediated immunity; praziquantel.

<sup>3</sup>Abbreviations used:

P-III-P = Procollagen-III-peptide

DTH = Delayed type hypersensitivity

SEA = *S. mansoni* soluble egg antigen

AWA = *S. mansoni* adult worm antigen

CD = Cluster of differentiation

95% CI = 95% confidence intervals

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## Introduction

Infections with *Schistosoma mansoni* have been associated with liver disease in Africa as early as 1904 (Symmers, 1904). Worldwide, schistosomiasis is the most frequent etiology of liver fibrosis (Warren, 1984). Portal hypertension, ascites and intestinal haemorrhages cause severe morbidity in Egypt and Brazil (Kamel et al., 1978; Andrade and Bina, 1983). In sub-saharan Africa, the clinical picture of hepatic schistosomiasis is less conspicuous, although liver enlargement is reported to occur in up to 15% of the infected individuals (Mahmoud, 1984). We have demonstrated that procollagen-III-peptide (P-III-P) and cholyglycine are sensitive markers for hepatic involvement in schistosomiasis due to *S. mansoni* in Brazil (Zwingenberger et al., 1988).

Impairment of cell-mediated immunity in human schistosomiasis is well known (Ottesen et al., 1978; Gastl et al., 1984) and the underlying numerical and functional alterations of lymphocyte subpopulations have been analysed (Colley et al., 1983; Feldmeier et al., 1985). Neopterin and  $\beta_2$ -microglobulin are serological indicators for abnormal lymphocyte activation in hepatosplenic schistosomiasis (Zwingenberger et al., 1988). The reversability of immunological alterations after specific chemotherapy has been demonstrated in intestinal and urinary schistosomiasis without hepatic fibrosis (Feldmeier et al., 1988). Clear evidence is still unavailable that chemotherapeutic elimination of adult worms in patients with hepatic schistosomiasis causes regression of immunopathology induced by eggs. We now investigated whether a) indicators of hepatosplenic disease previously defined in Brazilian patients are valid in an entirely different epidemiological situation in Zaire, and b) if they are useful criteriae to assess the reversability of hepatic schistosomiasis. The results indicate that serum cholyglycine, neopterin and  $\beta_2$ -microglobulin revert to levels close to normal two months after treatment with praziquantel. Serum concentrations of P-III-P rose shortly after treatment. A significant increase in the helper/inducer population is paralleled by augmentation of in vivo delayed type hypersensitivity.

## Material and Methods

### *Patients and controls*

The investigations were carried out in the Shaba province of Zaire, near the confluence of the Luvua and Lualaba rivers. Malaria is holoendemic in the region, and *S. haematobium* and *S. mansoni* occur in circumscribed foci. Neither loasis nor onchocerciasis occur in the area, and there is no clinical evidence for the presence of lymphatic filariasis. In the first two months of 1986, all patients with hepatomegaly presenting at the hospitals of Manono and Ankoro (n = 31) were examined. Infection by *S. mansoni* was the single cause for hepatomegaly evident in 22 persons, 12 males and 10 females with a median age of 13 years (range 5–60 years). This group with hepatic schistosomiasis will be referred to as "HS". The other nine patients (4 of them excreting *S. mansoni* ova), were excluded from the study, being homozygous for haemoglobin S (1); because etiology of hepatomegaly could not be elucidated (3) or since it was associated with active infection by hepatitis B or Epstein-Barr viruses

(5 patients, see below). 13 individuals infected with *S. mansoni* but without hepatomegaly (median age 11 years, range 5–50 years), detected during a parasitological survey, formed a second patient group (“IN”). 31 individuals from the same region matched for age (median 15, range 4–46 years) and sex (16 male, 15 female), in whom schistosomiasis was excluded by repeated parasitological examination, were used as controls (“CO”). From all subjects, a medical history was taken in French or, by the aid of local interpreters, in Kiluba. Special reference was heeded to potential liver noxes such as alcohol or consumption of staple foods possibly contaminated with aflatoxins. Previous medication was recorded. Hepatomegaly was assessed in cm in the midclavicular (MCL) and midsternal (MSL) lines. Criteria for exclusion from the study were: active infection by hepatitis B or Epstein-Barr viruses (HBs or HBe antigenaemia and/or IgM antibodies against HBc or VCA), sickle cell disease and evidence for alcohol abuse. All patients received 50 mg/kg praziquantel and were reexamined two months later.

#### *Parasitological methods*

Stool samples from three consecutive days were examined by the Kato-Katz technique (Katz et al., 1972); results were expressed as *S. mansoni* ova/g of stool. The presence of other intestinal helminths was noted. One sample of approximately 1 g was examined by MIF-concentration (Blagg et al., 1955). In addition, faecal smears were coloured by a modified cold Ziehl-Neelsen method to detect cryptosporidia (Snodgrass, 1984). For detection of microfilariae, buffy coats of 5 ml EDTA blood samples drawn between 10 and 12 h a.m. were filtered through polycarbonate membranes of 5 µm mesh size and stained as described (Feldmeier et al., 1981). Thick and thin blood films were prepared for the exclusion of acute malaria. The total micturition volume collected between 8 and 12 h a.m. was filtered for the detection of *S. haematobium* ova as described (Feldmeier et al., 1979). Proteinuria, haematuria, bilirubine and ketones were assessed by urine reagent strips (Combur, Boehringer Mannheim, FRG).

#### *Haematological methods*

EDTA blood was used for leucocyte and differential blood counts. The haematocrite was determined using a battery-operated microhaematocrite centrifuge. Erythrocytes were lysed and Hb-electrophoresis was performed as previously described (Bienzle et al., 1983).

#### *Immunological investigations*

Venous blood was collected between 8 and 12 h a.m. Sera were frozen immediately at  $-20^{\circ}\text{C}$  and transported to Berlin without thawing. There, aliquots were frozen at  $-70^{\circ}\text{C}$  without additives. For immunocytological staining, mononuclear cells (MNC) were separated by density gradient centrifugation on Ficoll-Hypaque (Biochrom, Berlin, FRG). 100.000 MNC suspended in 20 µl of phosphate buffered saline (PBS) containing 2% fetal calf serum (FCS) were sedimented onto the reaction fields of teflon-coated microscopic slides (Dunn, Asbach, FRG). The suspension was incubated for 10 min at room temperature in a moist chamber.

Then slides were gently rinsed with PBS/FCS three times and air dried rapidly with the aid of a ventilator. Control slides stained with Field's stain showed a distribution of MNC similar to that of cytocentrifuge preparations. The slides were stored at  $-20^{\circ}\text{C}$  until immunoperoxidase staining was performed.

T-lymphocyte phenotypes were differentiated by mouse monoclonal antibodies to CD 3, CD 4 and CD 8 (Behringwerke, Marburg, FRG). On each slide, four reaction fields were pre-incubated for 15 min with 25 µl of PBS containing 2% FCS and 1% pooled AB-serum. 25 µl of a 1:5 dilution of monoclonal antibodies in PBS was then added for 30 min at  $4^{\circ}\text{C}$ . One reaction field served as control with buffer only. Following three rinsings with PBS/FCS, 25 µl of peroxidase conjugated goat anti-mouse antibody (Tago, Burlingame, CA, USA) diluted 1:20 in PBS was added and incubated for a further 30 min at  $4^{\circ}\text{C}$ . Immediately after three rinsings with PBS/FCS, the substrate, 5 µl  $\text{H}_2\text{O}_2$  6% in 5 ml of diaminobenzidine, was layered on the slides and incubated for 10 min in the dark. Counterstaining was done by Mayer's haemalaun solution. Slides were read under 1000× magnifica-

tion, counting 200 lymphocytes per preparation. Commercially available radioimmunoassays were used to measure neopterin (Henning, Berlin FRG),  $\beta_2$ -microglobulin and total serum IgE (Pharmacia, Uppsala, Sweden), cholyglycine (Abbott, Chicago, USA) and procollagen-III-peptide (Behringwerke, Marburg, FRG). Increased levels of  $\alpha$ -foetoprotein were detected by radial immunodiffusion (Partigen, Behringwerke, Marburg, FRG). Antibodies to hepatitis viruses were tested by RIA (anti-HAV, anti HBs) or EIA (anti-HBc, anti-HBc/IgM, anti-HBe, HBeAg, HBsAg and anti-delta). Sera were tested for antibodies to Epstein-Barr virus components (anti-EA, -EBNA, -VCA and anti-VCA IgM) by indirect immunofluorescence. Sera were screened for antibodies to HIV-1 by ELISA (Abbott, Chicago, USA). None of the sera reacted in the ELISA, so 20 sera were chosen at random and tested by Western blot for antibodies to HIV-1 gp 41 and p 24. Antiplasmodial antibodies were assessed by indirect immunofluorescence using schizonts from in vitro culture of *P. falciparum* as antigen. Without exception, sera were reactive at 1:20 dilution. Titration was performed up to 1:1280. Antibodies against *S. mansoni* soluble egg (SEA) and adult worm (AWA) antigens were determined by an ELISA as previously described; results were expressed as antibody units (Feldmeier et al., 1983). In vivo delayed type hypersensitivity (DTH) was assessed by Multitest intradermal prick test (Institut Mérieux, Lyon, France), which combines seven recall antigens of ubiquitous distribution. After 48 h, only palpable induration exceeding the 2 mm diameter of the prick was counted; the sum of the diameters of all positive reactions is referred to as "score".

#### *Statistical analysis*

Medians and 95% confidence intervals (CI) were used to indicate means and dispersion of data. For the calculation of significance levels, non-parametrical tests were employed. The Mann-Whitney, the Wilcoxon matched pairs signed rank test and the Spearman's rank correlation coefficient test were applied when appropriate. Fischer's exact test was used to compare relative frequencies.

## **Results**

### *Clinical and parasitological findings*

Patient characteristics are summarized in Table 1. In HS patients, the liver width palpable below the right costal margin (MCL) ranged from 5 cm in a child up to 13 cm in one adult. The liver surfaces felt hard and moderately irregular upon palpation. Characteristically, the liver was enlarged in the MSL in all HS patients, reflecting predominant left lobe involvement.

In 4/13 IN patients and 5/31 controls, all under 9 years of age, the liver margin was palpable and of soft consistence. This finding, in children, was not considered abnormal. Upon reassessment two months after chemotherapy, no change exceeding 2 cm was noted. Ascites, present in six HS patients before treatment, was no longer detected in 4/6 patients reexamined after two months. None of the patients had a history of intestinal haemorrhage, whereas recurrent diarrhea and fever were reported in similar frequencies in all groups.

Before treatment, *S. mansoni* egg counts ranged from 10 to 2000 eggs per gram (median 84/g) in HS patients and from 10 to 264 (median 60/g) in IN patients. Two months after treatment with praziquantel, *S. mansoni* ova were still detected in stools of 3/23 HS patients; however, egg excretion had decreased from 54% to 98% in these individuals. Ova of *S. haematobium* were detected in 3 patients (range 1–21 ova/10 ml), persisting after therapy in one case. The prevalence of concomitant intestinal helminths was comparable in the patient

Table 1. Clinical characteristics of patients with schistosomiasis complicated by hepatomegaly (HS), intestinal schistosomiasis (IN) and local controls (CO) before treatment. Data indicate median and/or range

Group	HS	IN	CO
Number examined	22	13	31
Male/female	12/10	6/7	16/15
Age	13 (5–60)	11 (4–46)	15 (5–50)
History of*			
recurrent diarrhea	9/22	6/13	9/31
sanguinolent diarrhea	2/9	2/6	2/9
recurrent fever	12/22	9/13	13/31
ingestion of peanuts	11/22	6/13	14/31
heterozygous sickle cell trait	5/22	3/13	3/27
Liver MCL (cm)**	13–21****	9–11	9–12
Liver MSL (cm)***	6–12****	0–5	0–4
Spleen palpable	11/22****	1/13	2/31
(range in cm)	2–10	1	6–7

\* Relative frequencies of symptoms and risk factors for liver enlargement (consumption of peanuts and/or heterozygous sickle cell trait) did not differ between the three groups.

\*\* Width determined by percussion plus palpation.

\*\*\* Width palpable below the sternum.

\*\*\*\* Differences between HS patients and IN patients or controls are significant at  $p \leq 0.01$ .

groups and the control group (83%, 61% and 66%, respectively). Intestinal helminths found were *Ascaris lumbricoides*, *Trichuris trichiura*, *Strongyloides stercoralis*, *Hymenolepis nana* and hookworms. *Entamoeba histolytica* cysts were present in stools in 7% of HS and IN patients, respectively, and in 9% of controls. No cryptosporidia oocysts were detected. Microfilariae were not present in the daytime blood samples.

5/22 HS, 3/13 IN patients and 3/27 controls had the HbA/S genotype. These frequencies do not differ significantly. Proteinuria was observed neither in patients nor controls. Significant leucocyturia and erythrocyturia was observed in the urine of one patient with hepatomegaly.

### Serological findings

Evidence for past infection with the hepatitis B virus was detected in 83% and 77% of HS and IN patients, respectively, and in 72% of controls. None of the sera containing markers for hepatitis B infection contained antibodies to the delta hepatitis virus. Antibodies to hepatitis A virus were present in over 90% of all sera, as were antibodies to Epstein-Barr virus nuclear antigens (EBNA) and viral capsid antigen (VCA). Antibodies to EBV early antigen (EA) were present in two patients and in one control. None of the sera was positive for antibodies to HIV-1 by ELISA or Western blot analysis.

Table 2. Biochemical and immunological markers in patients with schistosomiasis complicated by hepatomegaly (HS), intestinal schistosomiasis (IN) and local controls (CO) before treatment. Data indicate median and 95% confidence intervals

Patient group . . . . .	HS	IN	CO	Differences
Number examined . .	23	13	31	between groups
Cholyglycine . . . . .	170	69	73.8	HS-IN $p \leq 0.05$
$\mu\text{g/dl}^*$ . . . . .	136–232	25–190	36–98	HS-CO $p \leq 0.05$
Procollagen-III-pep- tide . . . . .	10	8.7	8.4	HS-IN n.s.
$\text{ng/ml}^{**}$ . . . . .	9–13	8–9.7	7.8–9.4	HS-CO n.s.
$\beta_2$ -microglobulin . . . .	3.4	2.45	2.07	HS-IN $p \leq 0.05$
$\text{mg/dl}^{***}$ . . . . .	2.6–3.8	1.5–2.6	1.77–2.19	HS-CO $p \leq 0.001$
Neopterin . . . . .	12.55	11.65	8.1	HS-IN n.s.
$\text{nmol/l}^{***}$ . . . . .	11.5–14.2	9.1–16.1	7.5–9.0	HS-CO $p \leq 0.05$

\* Normal values established in northern hemisphere populations are 20–40  $\mu\text{g/dl}$  (Miller et al., 1981).

\*\* Normal values established in northern hemisphere populations are 2–12  $\mu\text{g/ml}$  (Rohde et al., 1979).

\*\*\* 2 mg  $\beta_2$ -microglobulin/dl and 10 nmol neopterin/l are considered as upper limits of normal in our laboratory.

The highest levels of  $\alpha$ -foetoprotein detected were 71 IU/ml in one patient with hepatomegaly, and 102 IU/ml in two patients with intestinal schistosomiasis and one control, respectively. In 57%, 55% and 65% of HS and IN patients and controls, respectively,  $\alpha$ -foetoprotein was not detected.

Antibodies to *Plasmodium falciparum* were detected in all patients. Median titres were 1:320 in all groups, and there was no significant difference between patients and controls.

In patients with hepatosplenomegaly, the median antibody response against AWA was 11.7 (95% CI, 3.9–16.3) antibody units; in controls, 2.2 (95% CL, 1.1–3.6). IN patients had intermediate values: median 7.9 (95% CI, 1.5–14.6) antibody units. Antibody response to SEA was marginally more pronounced in hepatomegalic patients (median 2.7 antibody units; 95% CI, 1.8–4.4) than in infections limited to the intestines (2.5; 1.0–3.4). Significant rises of antibody levels were encountered in HS patients two months after treatment: median anti-SEA antibodies rose to 5.0 (95% CI, 4.5–6.3;  $p \leq 0.02$ ), anti-AWA antibodies to 17.5 (95% CI, 8.0–46.0) antibody units ( $p = 0.01$ ).

As expected, the total serum IgE was elevated in HS (median 1340, 95% CI, 1180–1630 KU/l) and IN patients (2270; 1260–2774 KU/l). Controls had serum IgE levels commonly encountered in tropical surroundings (424; 286–632 KU/l).

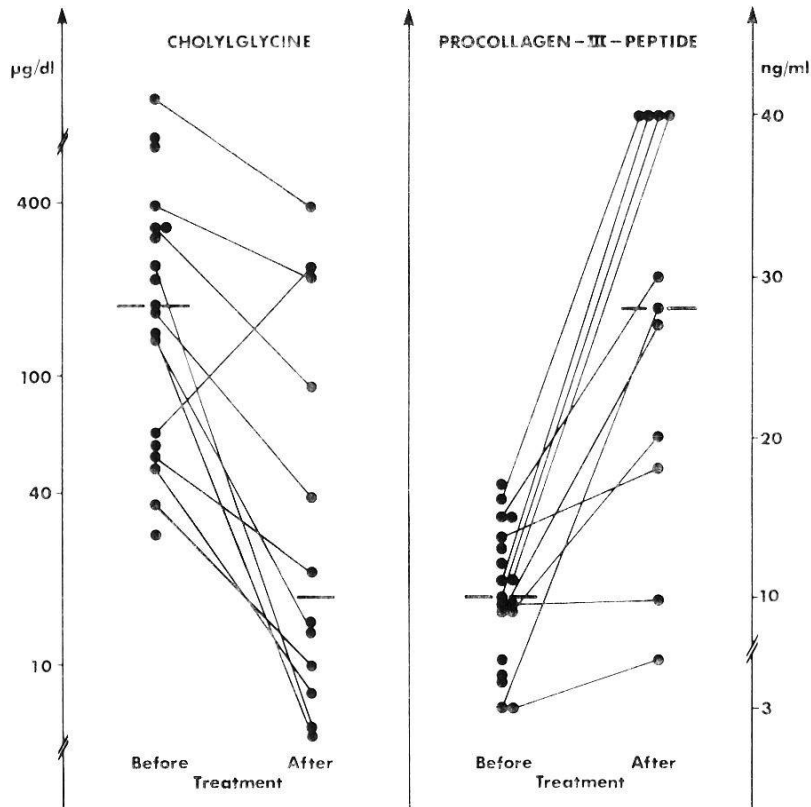


Fig. 1. Serum concentrations of cholyglycine and procollagen-III-peptide in schistosomiasis associated hepatomegaly before, and two months after chemotherapy with praziquantel. Bars indicate the medians. Changes after therapy were significant for cholyglycine ( $p \leq 0.01$ ) and procollagen-III-peptide ( $p \leq 0.001$ ).

### Biochemical findings

The pretreatment concentrations of cholyglycine, procollagen-III-peptide,  $\beta_2$ -microglobulin and neopterin in patients and controls are summarized in Table 2. Levels of cholyglycine and  $\beta_2$ -microglobulin were above normal values in the majority of subjects investigated, irrespective whether they belonged to patients or controls. However, the highest concentrations of these parameters were found in HS patients. Changes in cholyglycine and P-III-P concentrations following therapy are illustrated in Fig. 1. Surprisingly, only two months after treatment with praziquantel serum concentration of cholyglycine reverted almost to normal values ( $p \leq 0.01$ ). In contrast, the concentration of P-III-P rose significantly in HS patients ( $p \leq 0.001$ ), but did not change in IN patients (data not shown). Two months after therapy, neopterin levels in HS patients dropped to control values: median 8.4, 95% CI, 7.8–10 nmol/l ( $p \leq 0.05$ ).  $\beta_2$ -microglobulin decreased to 2.8 mg/dl (95% CI, 2.3–5.8 n.s.)

### T-cell subpopulations

The total number of lymphocytes, T-cells and T-cell subsets are summarized in Table 3. Total lymphocytes and pan-T-cells (CD3+) were decreased in



Table 3. Lymphocytes and lymphocyte subsets in the peripheral blood of patients with schistosomiasis associated hepatomegaly (HS), intestinal schistosomiasis (IN) and local controls (CO). Data indicate medians and 95% confidence intervals of the cell counts per  $\mu\text{l}$  blood

Patient group . . . . .	HS	IN	CO	Differences between groups
Number examined . . .	18	9	24	
Lymphocytes . . . . .	2050 1656–2510	3150 2250–3600	2965 2310–4000	HS–IN $p \leq 0.05$ HS–CO $p \leq 0.05$
T3 . . . . .	1498 1287–1735	2185 2100–3067	1670 1382–2314	HS–IN $p \leq 0.05$ HS–CO $p \leq 0.05$
T4 . . . . .	694 472–777	1400 1151–2004	1165 933–1600	HS–IN $p \leq 0.02$ HS–CO $p \leq 0.05$
T8 . . . . .	756 678–882	787 630–1035	491 311–1120	HS–IN n.s. HS–CO n.s.

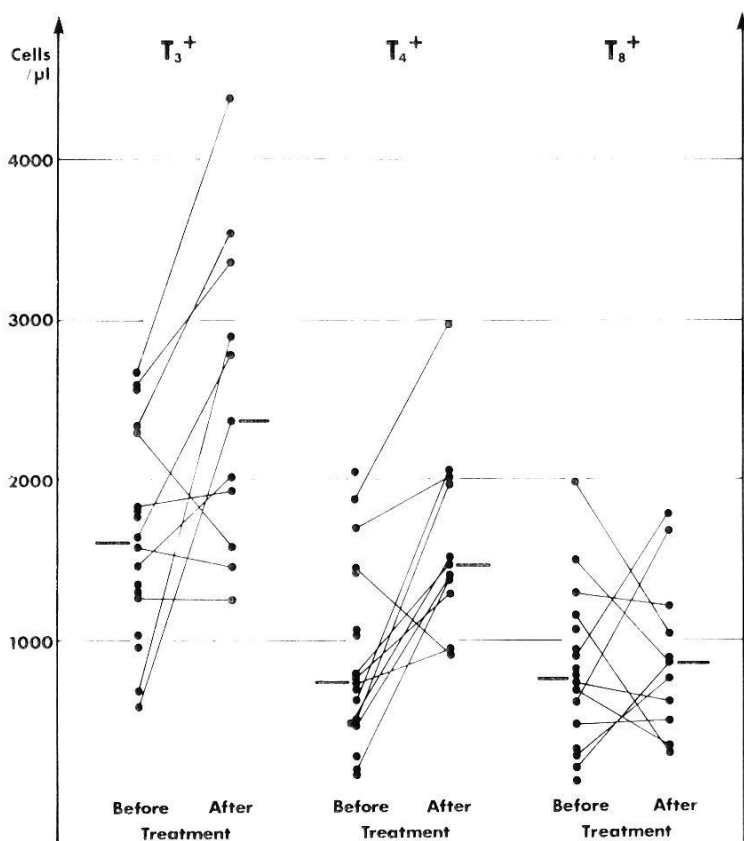


Fig. 2. T-lymphocyte subsets (cells/ $\mu\text{l}$  peripheral blood) before and two months after chemotherapy in patients with hepatic schistosomiasis. Median counts are indicated by the bars. Significance levels for post-therapeutic changes: T3  $p \leq 0.02$ ; T4  $p \leq 0.01$ ; T8 n.s.

patients with hepatomegaly as compared to patients with intestinal infection and controls ( $p \leq 0.05$ ). Within the T-cell compartment lymphocytes of the T helper/inducer (CD4+) subset were reduced in HS patients ( $p \leq 0.02$ ), whereas the number of T suppressor cells (CD8+) did not differ between the groups. After treatment with praziquantel, absolute lymphocyte counts rose to 3430/ $\mu\text{l}$

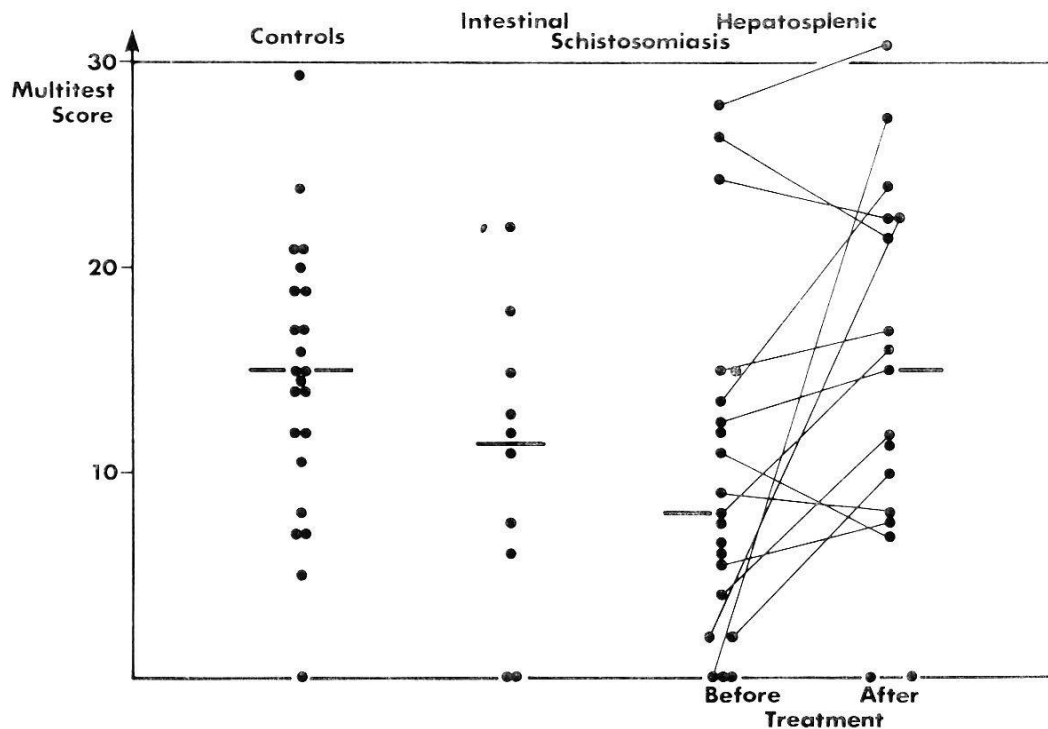


Fig. 3. Normalization of *in vivo* delayed type hypersensitivity after specific therapy of hepatosplenic schistosomiasis. Multitest scores (see methods) are shown for healthy controls (CO), patients with intestinal (IN), and hepatosplenic (HS) schistosomiasis before and for HS patients two months after therapy with praziquantel. Significance levels of differences before and for HS patients two months after therapy with praziquantel. Significance levels of differences before treatment: HS vs. CO:  $p \leq 0.05$ . IN vs. CO n.s. ( $p \leq 0.01$ ). After treatment with praziquantel the Multitest score increased significantly in HS ( $p \leq 0.02$ ), but not in IN patients (data not shown).

in HS patients (95% CI, 2950–4200;  $p \leq 0.02$ ). Within the T cell compartment, this increase was largely due to an increase of the CD4+ subset ( $p \leq 0.01$ ), whereas the number of CD8+ cells remained unchanged (Fig. 2).

#### *In vivo* delayed type hypersensitivity

*In vivo* DHS to recall antigens in the three study groups is illustrated in Fig. 3. Scores differed between hepatomegalic patients and controls ( $p \leq 0.05$ ), with intermediate scores in patients with intestinal schistosomiasis. After therapy, the median score in hepatomegaly patients rose by 40% ( $p \leq 0.02$ ). An inverse correlation between the Multitest scores and neopterin levels was observed in the HS group before ( $\rho = -0.48$ ;  $p \leq 0.05$ ), but not after treatment ( $\rho = -0.2$ , n.s.). No such correlation existed in IN patients or controls.

## Discussion

The effect of praziquantel in the treatment of hepatic schistosomiasis was assessed by biochemical and immunological parameters. Clinical observations from Brazil suggest that at least in some hepatomegalic patients antischistoso-

mal treatment leads to clinical regression of hepatosplenomegaly one to two years after administration of either oxamniquine or praziquantel (Bina and Prata, 1983). In the murine schistosomiasis model, a histopathological study recently demonstrated the reversability of fibrotic lesions after treatment with praziquantel (Morcos et al., 1985).

In a previous investigation, the immunological and biochemical parameters we selected to assess the effect of chemotherapy had been shown to reliably discriminate between cases of schistosomiasis associated liver fibrosis and disease limited to the intestinal tract. Serum cholyglycine, an indicator for spill-over of bile acids into the systemic circulation, was significantly elevated in the group with fibrosis. Remarkably, in two thirds of our patients with hepatomegaly cholyglycine levels had normalized two months after therapy. It is not clear, though, if hepatic bile acid clearance improved due to the recovery of previously impaired uptake by hepatocytes (Islam et al., 1985; Berry and Reichen, 1983), or because the portal blood flow previously distorted by collaterals (Miescher et al., 1983; Okhubo et al., 1984) normalized to a measurable degree during that short period. In human schistosomiasis, rationales exist that either of these mechanisms, or both, may increase cholyglycine concentration in peripheral blood: impairment of hepatic uptake as well as spill-over into the systemic circulation via porto-caval shunts. Clinical evidence for severe portal hypertension, however, was absent in our patients, since no episodes of haematemesis or melaena were reported. An additional determinant of bile acid clearance, the sinusoid endothelial lining, is characteristically altered in schistosomiasis mansoni (Grimaud and Borojevic, 1977). The microvilli of the vascular pole of hepatocytes are flattened, while the sinusoid endothel includes pathological fibrillary layers which resemble a basement membrane.

Before treatment, serum levels of procollagen-III-peptide were marginally higher in patients with hepatic schistosomiasis than in IN patients and controls, and fell within normal ranges previously published for African individuals (Bolarin et al., 1984). Surprisingly, P-III-P concentrations increased significantly two months after treatment (Fig. 1,  $p \leq 0.001$ ). Studies using antibodies directed against P-III-P on liver biopsies may provide an explanation. In vitro, cleavage of the N-terminal propeptide of type III collagen occurs during the secretion of procollagen into the extracellular compartment. However, there is evidence that, in precipitated collagen formation, the collagen type III precursor molecule is integrated into tissue collagen uncleaved, that is, still containing the N-terminal P-III-P (Wick et al., 1978). When monoclonal anti-procollagen type III antibodies were applied to liver sections in murine schistosomiasis mansoni, dense deposits of procollagen type III were demonstrated containing the N-terminal propeptide entity in situ (Parise et al., 1985).

In a separate investigation, we found evidence that the net stimulation of components of the macrophage-lymphocyte system in schistosomiasis is measurable by serum neopterin and  $\beta_2$ -microglobulin (Zwingenberger et al., 1988).

$\beta_2$ -microglobulin is increasingly released during the membrane turnover of activated lymphocytes (Cresswell et al., 1974), whereas neopterin is secreted by T-cell activated macrophages (Huber et al., 1985). 75% of our controls had neopterin concentrations  $\leq 10$  nmol/l, indicating that, in the absence of concomitant infections such as malaria, neopterin production may be attributed to macrophage activation in response to schistosome antigens. Contrarily, all  $\beta_2$ -microglobulin levels measured are high by European standards. Furthermore, neopterin but not  $\beta_2$ -microglobulin levels receded after treatment with praziquantel.

Absolute lymphocyte counts of both patient groups and controls were within normal ranges. However, the relative diminution of the T helper/inducer subset in HS patients is consistent with previous reports in patients with hepatosplenic schistosomiasis mansoni (Colley et al., 1983), and in children with severe mixed *S. mansoni* and *S. haematobium* infections (Feldmeier et al., 1985). After therapy, a slight rise in absolute lymphocyte counts occurred. Within the T-cell compartment, this rise was exclusively due to cells of the CD4+ subset, while the number of CD8+ positive cells remained unchanged ( $p \leq 0.001$ ). Accordingly, CD4+ counts in HS patients no longer differed from those of controls two months after treatment. The augmentation of the CD4/CD8 lymphocyte ratio was impressively paralleled by the normalization of in vivo delayed type hypersensitivity as assessed by the Multitest Mérieux. Two months after therapy with praziquantel, 12/15 HS patients had scores within the normal range. In this context, it is worth mentioning that, by standardization of intradermal antigen application, global comparability of results was achieved. Multitest scores in Zairean patients with hepatomegaly, and those in controls, were congruent to the median scores in patients with hepatosplenic schistosomiasis and healthy individuals, respectively, in Brazil (Zwingenberger et al., 1988). The increase of Multitest scores after antischistosomal therapy suggests a causal relation of altered in vivo cell-mediated immune responses to the parasitic infection, and that specific chemotherapy paves the way for the normalization of cellular immune responsiveness.

The notion that hepatomegaly in our patients was predominantly due to schistosomal infection is supported by the strict admission criteria and by the fact that other factors liable to contribute to liver enlargement were no more prevalent in patients than controls. This includes malaria as assessed by indirect immunofluorescence, infections with hepatitis A, B, delta and Epstein-Barr viruses, and heterozygosity for the sickle cell trait. Pathological  $\alpha$ -foetoprotein levels were detected in equal frequency in both patient groups and controls. However, discrete elevations of  $\alpha$ -foetoprotein have been described in various noncarcinomatous conditions including viral hepatitis and schistosomiasis mansoni (Bout et al., 1982).

The importance of this study is manifold. Comparison of results to those of a previous study from Brazil indicate that concentrations of cholyglycine and

neopterin in serum and alterations of T-cell phenotype frequencies characterize patients with hepatic schistosomiasis, even if they live under completely different endemic conditions. Combining the biochemical and immunological parameters, staging of the dynamic pathophysiological process occurring in individual patients may be feasible. Elimination of adult worms by treatment with praziquantel is associated with diminution of egg induced immunopathology as early as two months after administration of the drug, at a time when clinical assessment of liver and spleen size and morphology is unreliable for the measurement of regression of the disease.

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