

## IMMUNE COMPLEXES IN SCHISTOSOMIASIS

### V — CHARACTERIZATION AND QUANTITATION OF THE IMMUNOGLOBULINS PRESENT IN IC

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#### SUMMARY

Ig classes were investigated in 3% polyethylene glycol (PEG) precipitates of sera from patients infected by *Schistosoma mansoni*. IgG and IgE were demonstrated in 100% of the cases, IgM in 83%, and IgA in 79%. In our experimental conditions, IgD was not found in these precipitates. A direct correlation was demonstrated between IgM levels in 3% PEG precipitates and circulating IC levels.

#### INTRODUCTION

Circulating immune complexes (IC) due to infection by *Schistosoma mansoni*, were previously demonstrated by the Authors<sup>11,12</sup> in patients and mice infected by this parasite. These IC, probably present in circulation before their possible deposition, were detected and quantified by several methods<sup>3,8,13,14</sup>. A relationship was observed between IC levels and clinical forms of the schistosomiasis<sup>11</sup>. Immunoglobulins belonging to the G, M or E classes, as well as the specific *Schistosoma* antigen called "number 4" were found in these IC<sup>2</sup>. Recently, we demonstrated the existence of an inverse correlation between C<sub>3</sub> component of the complement and IC levels<sup>10</sup>. In the same work, C<sub>3</sub> and C<sub>4</sub> and C<sub>1q</sub> were characterized in the circulating IC.

The purpose of this work was to quantify and compare IgG, IgM, IgA and IgE levels in 3% PEG precipitates, and circulating IC levels.

#### MATERIAL AND METHODS

Sera were obtained from 29 patients with chronic schistosomiasis from a hyper-

endemic area of Bahia State (Brazil). *Schistosoma mansoni* infection was confirmed by the detection of viable eggs in the stool and by serological tests (immunoelectrophoresis and hemagglutination). All patients had hepatointestinal form of schistosomiasis. Control sera were obtained from 9 healthy subjects.

Radiolabeled human IgE was obtained from PHARMACIA (Uppsala-Sweden).

Aliquots of patient and control sera were precipitated with 3% polyethylene glycol (PEG) according to CREIGHTON et al.<sup>3</sup> as modified by the Authors<sup>11</sup>. The same method was employed to study the solubility of IgE in PEG.

IgG, IgM, IgA and IgD were measured in 3% PEG precipitates by radial immunodiffusion as described by MANCINE et al.<sup>7</sup> on plates supplied by the BEHRING Institute (Harburg-Lohn-W, Germany).

Quantitation of IgE was performed by the radioimmunosorbent test (Kit Phadebas — PHARMACIA — Uppsala — Sweden).

Radiolabeled C<sub>1q</sub> binding test was performed according to NYDEGGER et al.<sup>8</sup>.

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Results were statistically analyzed by a test of correlation. The distribution of Ig levels not being gaussian, logarithms of Ig concentrations were calculated for the test. The chi square test was used to compare the frequencies and the levels.

## RESULTS

The presence of IgG, IgM, IgA and IgD was investigated by radial immunodiffusion in the 3% PEG precipitates of sera from 29 infected patients and 9 controls. The frequency of these immunoglobulins is shown in Table I. The mean level of the immunoglobu-

lins present in the precipitates is shown in Table II. A statistically significant difference was observed in both frequency and level of IgG, IgM and IgA found in the 3% PEG precipitates of sera from infected patients compared to those from controls ( $p < 0.01$ ). In our study, IgD was not found in any precipitate.

Radioimmunoassay of IgE was carried out in 3% PEG precipitates of sera from 10 patients infected with *S. mansoni* and 6 controls. The frequency of characterization and the mean level of this Ig are shown in Table III. A significant amount of IgE was found in IC from infected patients.

T A B L E I

Immune complexes in schistosomiasis. Frequency of the characterization of Ig classes in 3% PEG precipitates.

Subjects	Number of cases	IgG	IgM	IgA	IgD
Infected patients	29	29 (100%)	24 (82.75%)	23 (79.3%)	0
Controls	9	1 (11.1%)	4 (44.4%)	2 (22.2%)	0

T A B L E II

Immune complexes in schistosomiasis. Levels of Ig classes (IU/ml) present in 3% PEG precipitates.

Subjects	Number of cases	IgG	IgM	IgA
Infected patients	29	6.48 ± 1.3	29 ± 14.1	10.8 ± 5.8
Controls	9	0.21 ± 0.63	13.7 ± 16.5	2.7 ± 5.3

T A B L E III

Immune complexes in schistosomiasis. Frequency of the characterization and levels of IgE presents in 3% PEG precipitates.

Subjects	Number of cases	IgE	
		Frequency	Mean (IU/ml)
Infected patients	10	10 (100%)	35.84 ± 40.5
Controls	6	3 (50%)	1.40 ± 1.01

The levels of immunoglobulins present in 3% PEG precipitates of infected patients' sera were compared to circulating IC levels evaluated by the  $C_{1q}^{-125}I$  binding test. Statistical analysis showed a correlation between the le-

vels of IgM and circulating IC ( $r = 0.55412$ ;  $df = 27$ ;  $0.001 < p < 0.01$ ) (Fig. 1). In our study, no correlation was observed between the levels of IgG, IgA or IgE in 3% PEG precipitates and the quantities of circulating IC.

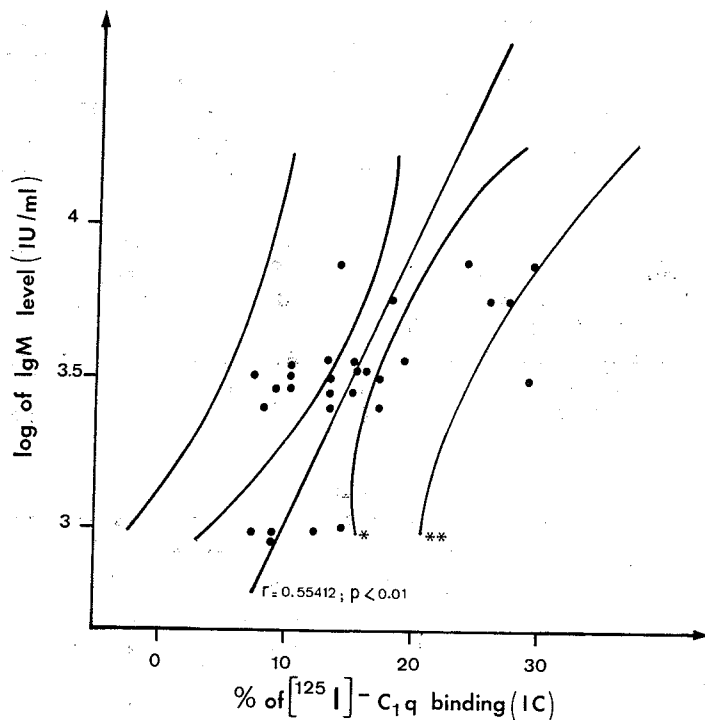


Fig. 1: Immune complexes in schistosomiasis. Correlation between IgM present in 3% PEG precipitates and IC levels.

\* confidence limits for the mean

\*\* confidence limits for the population

## DISCUSSION

This study demonstrated the presence of different immunoglobulin classes in circulating IC from patients infected with *S. mansoni*. IgG and IgE were found in 100% of the cases, IgM in 83% and IgA in 79%. The absence of IgM and IgA in some cases and the presence of IgE in all the cases studied, could be due to the higher sensitivity of radioimmunoassay used to quantify IgE in 3% PEG precipitates compared to radial immunodiffusion used to measure the other immunoglobulin levels. In the control group IgG was found in 11%, IgE in 50%, IgM in 44% and IgA in 22% of the cases studied. The sig-

nificant difference between the frequency and levels of these immunoglobulins in 3% PEG precipitates of sera from infected patients compared to controls permits us to conclude that this precipitation method can be used to separate IC and free immunoglobulins. In fact, CREIGHTON et al.<sup>3</sup> have shown that free human IgG, IgM and IgA do not precipitate in a PEG concentration lower than 5%. HOUBA & LAMBERT<sup>6</sup> have confirmed that IgM is soluble in a PEG concentration solution lower than 5%. The concentration of PEG employed in this work was only 3%. The study of IgE solubility in different PEG concentrations (Fig. 2) reveals that unaggregated IgE does not precipitate in less than 8% PEG.

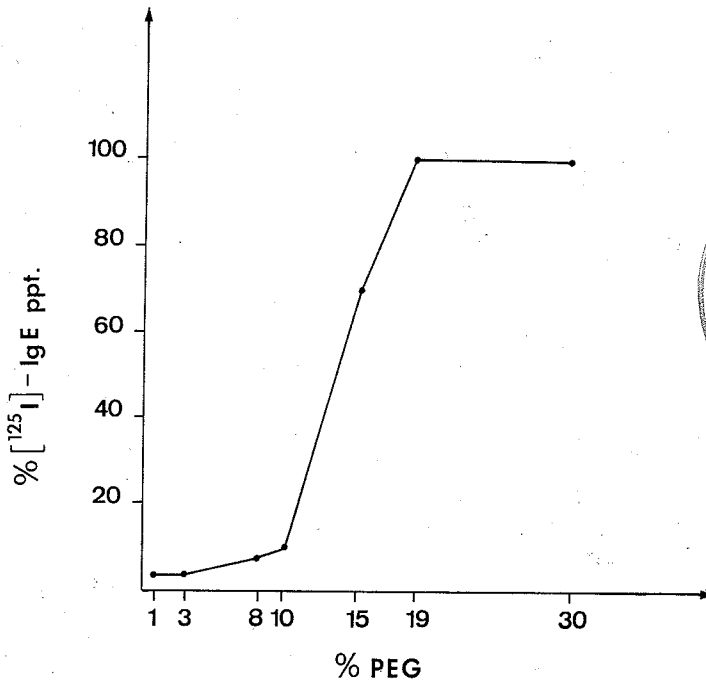


Fig.2: Solubility of human IgE in various concentrations of PEG and in the presence of normal human serum

In a previous work<sup>2</sup>, IgA was not detected by a double diffusion method in 3% PEG precipitates of infected patients' sera. This was probably due to the lower sensitivity of the OUCHTERLONY method<sup>9</sup>.

The high level of IgE in 3% PEG precipitates from infected patients' sera suggests its participation in IC. These "IgE-Ag" complexes might play an important role in the "immunological trigger mechanism of IC deposition" as described by BENVENISTE<sup>1</sup>. The characterization of specific IgE antibodies in schistosomiasis<sup>4</sup> and recently the demonstration of IgE deposits in kidneys from patients infected with *S. mansoni*<sup>5</sup> are arguments in favour of their participation in circulating IC due to *S. mansoni*.

An interesting correlation was observed between levels of IgM present in PEG precipitates and amounts of circulating IC. This

correlation permits us to propose IgM quantitation in 3% PEG precipitates as an indirect method of IC detection in serum. In our study, no correlation was found between the quantities of other immunoglobulins and IC levels. This may be due to the presence of smaller quantities of complexed IgG in the 3% PEG precipitates from infected patients' sera. It is possible that IgG, when complexed to a low molecular weight antigen, does not precipitate in a 3% PEG concentration. In contrast, IgM complexed to the same antigen, can be precipitated by taking advantage of its high molecular weight. The absence of any correlation between the IgA and IgE levels in PEG precipitates, and circulating IC, evaluated by the C<sub>1q</sub> binding test, can be explained by the fact that the first complement component does not bind to their IC.

## RESUMO

### Imuno-complexos na esquistossomose

#### V — Caracterização e dosagem das imunoglobulinas presentes no IC.

As classes de imunoglobulinas foram investigadas nos precipitados em PEG à 3% dos soros de esquistossomóticos. IgG e IgE foram demonstradas em 100% dos casos, IgM em 83% e IgA em 79%. Nas nossas condições experimentais, IgD não foi encontrada nesses precipitados. Uma correlação diretamente proporcional foi demonstrada entre as taxas de IgM detectadas nos precipitados em PEG à 3% e a dosagem dos IC circulantes.

### ACKNOWLEDGMENTS

We would like to thank Mrs. Fabienne DERBAUDRENHIEU and Mrs. Marie-Christine LIEBART for their excellent technical assistance in this work.

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Recebido para publicação em 17/3/1976.