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IMMUNE COMPLEXES IN SCHISTOSOMIASIS

IV — C_3, C_4 and C_{1q} characterization and correlation between C_3 in serum and circulating IC levels

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SUMMARY

C_3, C_4 and C_{1q} fractions of complement (C') were investigated in 3% polyethylene glycol (PEG) precipitate of sera from **Schistosoma mansoni** infected patients. C_3 was characterized in 96.8%, C_4 in 90.3% and C_{1q} in 19.3% of the cases studied. An inversely proportional correlation was demonstrated between C_3 and IC levels in serum.

INTRODUCTION

The discovery of the C_3 component of the complement (C') in the granular deposits on the glomerular basement membrane of patients with hepatosplenic schistosomiasis [7,15] was very important for the better understanding of the nature of these deposits. IgG and IgM characterizations in glomerulus[7,15] and the recent discovery of a **S. mansoni** specific antigen in a kidney eluate [7] have contributed considerably to the hypothesis that the antigen-antibody complexes are directly responsible for the patients' nephropathy [1,2,4,5,7,11,15].

In previous studies, the Authors have detected and characterized, by different methods, circulating Immune Complexes (I. C.) in sera from **S. mansoni** infected patients [3,13,14]. In addition to immunoglobulins G, M and E, we have shown the existence of number "4" specific **Schistosoma** antigen in the IC [3]. In a more recent work, the Authors quantified IgG, IgM, IgA and IgE present in IC, obtaining a directly proportional correlation between the quantities of complexed IgM and circulating IC [12].

In this study, the Authors have investigated the C' in circulating IC, and tried to correlate the quantities of C_3, C_4 and C_{1q} to the IC levels.

MATERIAL AND METHODS

Sera

Sera from 31 carriers of chronic schistosomiasis were used in this study. Infection by **S. mansoni** was verified by the detection of viable eggs in feces and by serological tests for the diagnosis of schistosomiasis. As control, normal human sera (NHS) of 10 individuals free from any parasitic or microbian infection were used. The anti-C_{1q} serum was obtained from the Hyland Laboratory. Anti-C_3 and anti-C_4 immunesera were included in the BEHRING plates.

Methods

Precipitation with polyethylene glycol (PEG) described by CREIGHTON et al. [6] and modified by the Authors [13], was used to isolate the IC from the other serum components.

C_3 and C_4 were quantified in serum and characterized in 3% PEG precipitated by radial immuno-diffusion as described by MAN-

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CINI et al. [9] on plates supplied by the BEHRING Institute. The same method [9], was used to quantify the C_{1q} present in serum and to characterize it in 3% PEG precipitates.

The C_{1q}-^{125} I binding test was done according to NYDEGGER et al. [10].

Results were statistically analyzed by the correlation test establishing a rate of regression over their logarithmical coordinates, and by the chi-square test.

RESULTS

The presence of C_3, C_4 and C_{1q} in the PEG precipitates of sera from 31 infected patients and 10 controls was studied by radial immunodiffusion [9]. The frequency of these constituents is shown in Table I.

TABLE I

Immune complexes in schistosomiasis. Characterized C_3, C_4, and C_{1q} in 3% PEG precipitates

Subjects	Number of cases	C3	C4	C1q
Infected patients	31	30 (96.8%)	28 (90.3%)	6 (19.35%)
Controls	10	4 (40%)	1 (10%)	0

A statistically significant difference was observed in the frequency of C_3 and C_4 detected in the 3% precipitates of sera from patients compared to controls ($p < 0.01$).

Regarding C_{1q}, although all controls were negative, there is no significant difference, because of its weak positivity in 3% PEG precipitates of serum from infected patients.

C_3, C_4 and C_{1q} in the serum were quantified by radial immunodiffusion [9], and compared to circulating IC levels evaluated by the C_1-^{125}I binding test [10]. A statistical analysis permitted us to observe an inversely proportional correlation between levels of C_3 and circulating IC ($r = 0.8238$; $p < 0.001$) (Fig. 1).

In our experimental conditions, no correlation was found between C_4 and C_{1q}, and circulating IC levels.

DISCUSSION

The results above show that of 31 infected patients, 30 (97%) presented C_3, 28 (90%) presented C_4, and 6 (19%) presented C_{1q} in the 3% PEG precipitates. In comparison, the respective percentages in the PEG precipitates of the control group were 40%, 10% and 0%. A significant difference in the frequency of C_3 and C_4 in sera from infected patients compared to controls permits us to conclude that it is probably due to the presence of C_3 and C_4 in circulating IC.

However, our work shows for the first time in schistosomiasis, an inversely proportional correlation between levels of C_3 and circulating IC. In other words, the higher the level of circulating IC, the lower the serum C_3 and vice-versa. A similar relationship was demonstrated by HUNDER & MC DUFFIE [8] and WATTRE et al. [16] in rheumatoid arthritis. This correlation suggests the existence of an important consumption of C_3 by IC in patients'serum. In our experimental conditions, no correlation was observed between C_4 and C_{1q}, and IC levels. Two hypotheses may be proposed, excluding an occasional association, for a possible explanation of this phenomenon. Firstly activation of both classical and alternate complement pathways could be admitted. The classical pathway would be assured by the demonstration in this study of complement components in circulating IC from infected patients'serum. The methods of C_{1q} binding and complement fixation, used to detect IC, also represent the classical pathway. Alternate pathway activation could also occur. In previous works, the Authors [3,13] have demonstrated the participation of IgE and IgA in circulating IC from schistosome infected patients's serum. When linked to an antigen, these immunoglobulins may activate the alternate pathway. C_{1q} and C_4 do not participate in this activation, and are therefore not consumed. In contrast, C_3 is very much implied.

On the other hand, C_{1q} is known to have a very labile linkage with the Fc fragment of a complexed immunoglobulin. In fact, it does not attach itself definitively to Ag-Ab complexes. In this manner the first C_1 component

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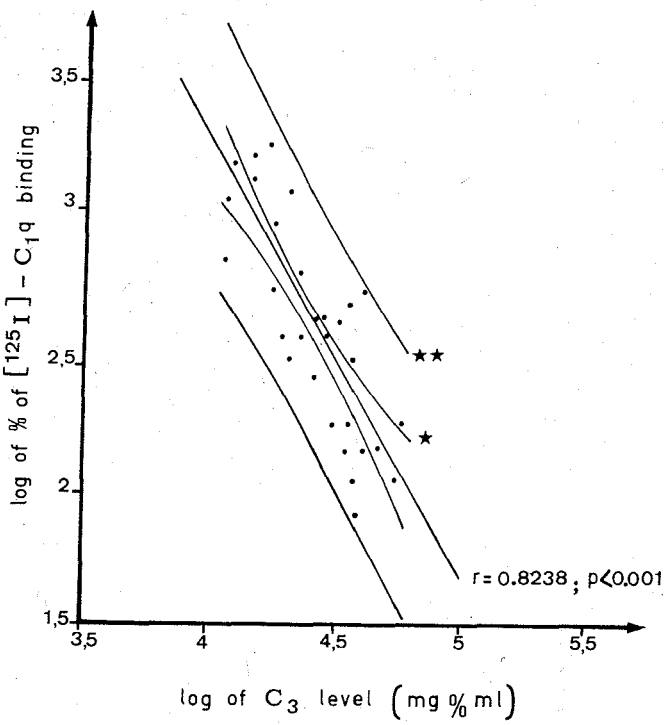

Figure 1 : IMMUNE COMPLEXES IN SCHISTOSOMIASIS CORRELATION BETWEEN C_3 AND IC LEVELS

★ confidence limits for the mean

★★ confidence limits for the population

may be used several times in the classical complement pathway activation. Likewise C_4, together with C_2, participating in a decisive phase of the classical pathway activation, is not fixed definitively to the C' system, when this is linked to the IC. On the contrary, C_3 can be fixed definitively, in any pathway of complement activation.

Although the C' activation can frequently exist in the circulation, as observed in this work, it is possible to foresee a local activation of this system, which would play an important role in renal pathology, specially with the inflammatory phenomena that may be caused by the enzymes freed.

In the future, it could be interesting to carry out a more complete study, including the alternate pathway of activation, which probably has an important role in the mechanisms that take place in the IC deposits.

RESUMO

Imuno-Complexos na esquistossomose. IV — Caracterização do C_3, C_4 e C_{1q} e correlação entre as taxas do C_3 Sérico e os IC Circulantes.

As frações C_3, C_4 e C_{1q} do complemento (C') foram investigadas nos imuno-complexos (IC) circulantes precipitados pelo polietilenoglycol (PEG) do soro de 31 esquistossomóticos. C_3 foi caracterizado em 96,8% dos casos, C_4 em 90,3% e C_{1q} em 19,3%.

Demonstrou-se uma correlação inversamente proporcional entre as taxas séricas de C_3 e IC.

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