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Complement activation in patients with Gambian sleeping sickness

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SUMMARY

Low levels of complement components C3, C4 and factor B were found in the sera of most patients with early and advanced Gambian trypanosomiasis. C3 levels increased after treatment with melarsoprol (Mel B). Nearly all patients had high serum IgM levels and a significant correlation was found between high serum IgM levels and low serum C3 levels. High concentrations of cryoglobulins, which contained IgM and C3, were found in most of the sera tested. Formation of large molecular weight IgM complexes may play an important part in producing the low C3 levels found in patients with Gambian sleeping sickness.

INTRODUCTION

The demonstration of high immunoconglutinin titres in experimental and human trypanosomiasis (Ingram & Soltys, 1960; Pautrizel *et al.*, 1962) suggests the occurrence of complement activation in this disease. More direct evidence for complement activation in experimental trypanosomiasis has recently been presented by Nagle *et al.* (1974) who found low serum C3 and C4 levels in monkeys infected with *Trypanosoma brucei rhodesiense*. Immune complexes found in the renal glomeruli of these monkeys contained properdin, suggesting activation of the alternative complement pathway.

The pathogenesis of human sleeping sickness is still not fully understood but vascular damage probably plays an important part in producing the neurological features of the disease. Complement activation, perhaps as the result of immune complex formation, could be playing a part in the production of this vascular damage. We have therefore studied some aspects of the complement system in sixty patients with Gambian sleeping sickness and have found evidence of complement activation in most patients with the disease.

PATIENTS AND METHODS

Patients. Sixty patients with Gambian trypanosomiasis seen at the field station of the Nigerian Institute for Trypanosomiasis Research, Gboko or at Ahmadu Bello University Hospital, Zaria, Nigeria were studied. A diagnosis of trypanosomiasis was made by the detection of trypanosomes in gland juice or cerebrospinal fluid (CSF) or by the detection of a high CSF IgM in a patient with clinical features suggestive of sleeping sickness. Thirteen patients had early disease without involvement of the nervous system, the remainder had advanced disease with laboratory evidence of central nervous system involvement. Eleven of the patients with advanced disease still had a positive gland puncture. None of the patients had any clinical features of immune complex disease. Cutaneous vasculitis, arthritis and iridocyclitis were not seen. Only two of the twenty-five patients tested had proteinuria and one of these patients had schistosomiasis. All had normal blood ureas.

All patients were treated with melarsoprol (Mel B). Patients with early disease received a single course, those with advanced disease two or three courses. Each course consisted of three injections of 3·6 mg/kg body weight. Some patients with advanced disease received suramin before Mel B.

Forty healthy adult Nigerians acted as controls for the complement determinations.

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Methods. Serum C3, C4, factor B and IgM levels were measured by radial immunodiffusion using monospecific antisera (Hyland Laboratories and Dutch Red Cross Blood Transfusion Laboratory, Amsterdam). Complement levels are expressed as a percentage of a pooled adult Nigerian serum standard; IgM levels are recorded in international units. Sera from patients and controls were stored for at least 1 month before they were tested.

Cross-over immunoelectrophoresis was carried out on plasma samples collected with EDTA. Agarose at a concentration of 0·75% in Tris–barbital buffer, pH 8·8 and ionic strength 0·05, containing 0·01 M EDTA was used as the supporting medium. The initial run was carried out at 200 V for approximately 4 hr. The second run was carried out overnight at a constant current of 4 mA per glass plate. A water-cooled platen was used for both runs.

Sera for cryoglobulin determination were allowed to stand at 4°C for 1 week. Precipitates were sedimented by centrifugation at approximately 10,000 *g* using the high-speed head of an MSE 2 L centrifuge. Precipitates were washed six times in cold phosphate buffered saline (PBS) and then redissolved in 0·5 ml of PBS at 37°C. The protein content of each cryoglobulin solution was measured and the nature of the cryoglobulin determined by immunodiffusion in 1·5% agar against antisera to IgA, IgG, IgM, C3 and a trypanosome antigen. The trypanosome antiserum was raised in rabbits by immunization with a preparation obtained from sonicated *T. b. gambiense* emulsified in complete Freund's adjuvant. Cryoglobulins were tested for rheumatoid factor using a slide latex test.

RESULTS

Initial complement levels

The serum C3, C4 and factor B levels found in sixty patients with Gambian trypanosomiasis at the time of their presentation at hospital are shown in Figs 1–3. Patients with early or advanced disease had significantly lower C3, C4 and factor B levels than the controls ($P = <0{\cdot}001$ for each comparison). C3 and C4 levels were more markedly depressed than those of factor B. Patients with early disease had lower mean levels of C3, C4 and factor B than patients with advanced disease and a negative gland puncture but the difference between the two groups is only statistically significant for C3 ($P = <0{\cdot}01$).

Cross-over immunoelectrophoresis of EDTA plasma from six patients with low C3 levels on radial immunodiffusion showed the presence of C3 breakdown products in 3 (Fig. 4). Cerebrospinal fluid from six patients with advanced disease was concentrated about fifty-fold by negative pressure dialysis and tested for complement breakdown products by cross-over immunoelectrophoresis but none were found.

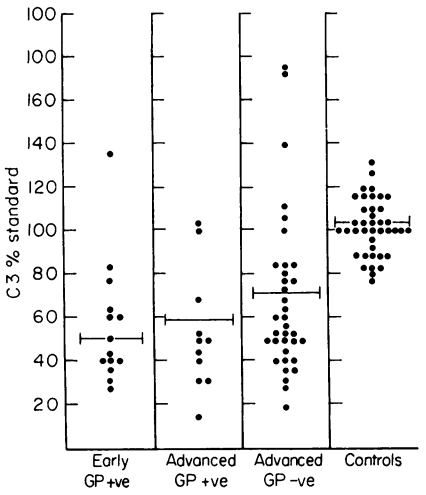

FIG. 1.

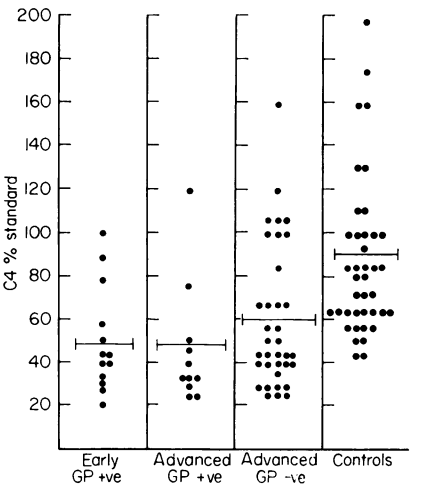

FIG. 2.

FIG. 1. Mean and distribution of serum C3 levels in sixty patients with Gambian sleeping sickness and in forty controls. GP = Gland puncture.

FIG. 2. Mean and distribution of serum C4 levels in sixty patients with Gambian sleeping sickness and in forty controls. GP = Gland puncture.

The response of C3 levels to treatment

C3 levels were measured in fifteen patients (five with early disease and ten with advanced disease) before and 2 weeks after the start of treatment, by which time patients with early disease had received one course of Mel B and those with advanced disease two courses of Mel B. In nearly all patients a rise in serum C3 occurred following treatment (Fig. 5) ($P = <0{\cdot}01$).

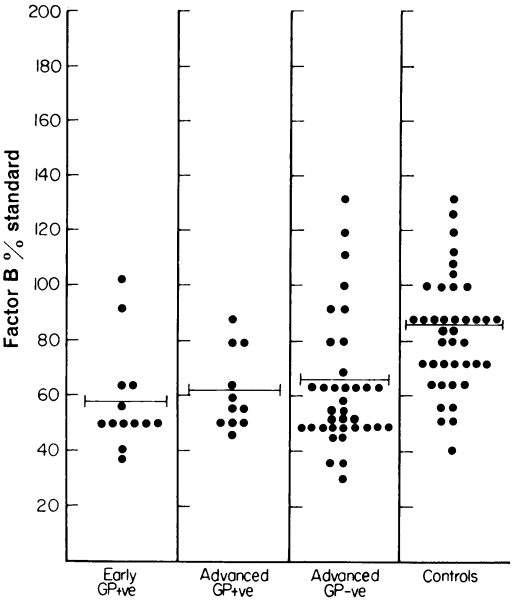

FIG. 3. Mean and distribution of serum factor B levels in sixty patients with Gambian sleeping sickness and in forty controls. GP = Gland puncture.

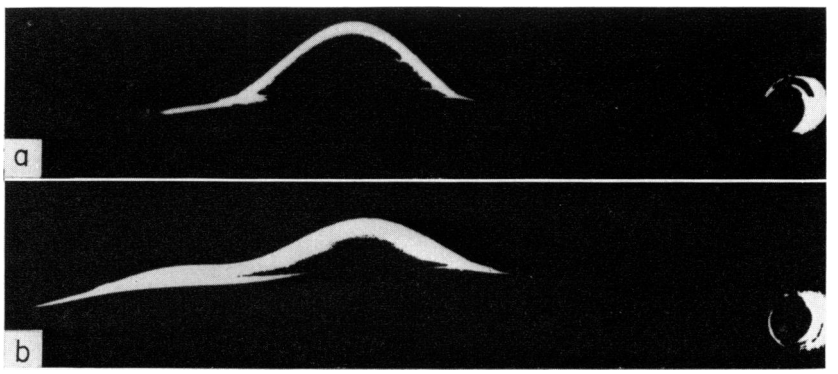

FIG. 4. Cross-over section immunoelectrophoresis showing the presence of altered C3 in the plasma of a patient with Gambian sleeping sickness. (a) Control plasma, (b) patient's plasma.

Serum IgM and C3 levels

Serum IgM was elevated in most patients. However, the mean level for patients with early disease (1250 i.u./ml $\pm$ 470 i.u./ml) was significantly higher than the mean level of patients with advanced disease (846 i.u./ml $\pm$ 506 i.u./ml) ($P = <0{\cdot}01$). In both groups some correlation was found between the presence of a high serum IgM and a low serum C3 ($r = 0{\cdot}44$, $P = 0{\cdot}01$). This correlation was most marked for patients with the highest serum IgM levels (Fig. 6).

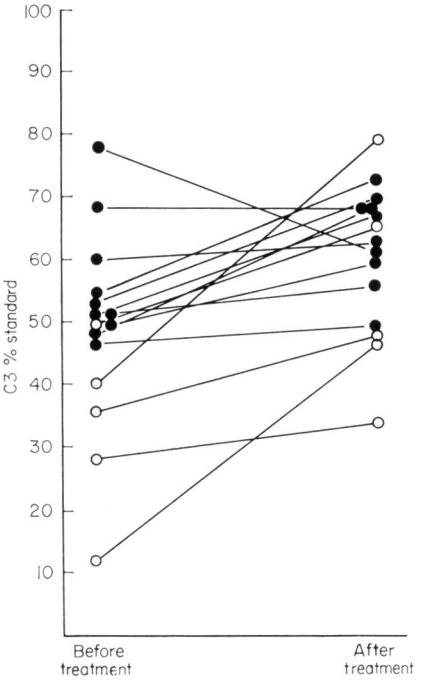

FIG. 5. Serum C3 levels in fifteen patients with Gambian sleeping sickness before and after treatment with Mel B. (○). Patients with early disease, (●) patients with advanced disease.

Serum cryoglobulins and C3 levels

Nine of eleven patients with advanced trypanosomiasis had serum cryoglobulin levels above the highest value recorded in sera from healthy controls (Fig. 7). All cryoglobulins contained IgM and most contained C3 (Table 1). Trypanosome antigen could not be detected. A significant correlation was found between high serum cryoglobulin concentrations and a

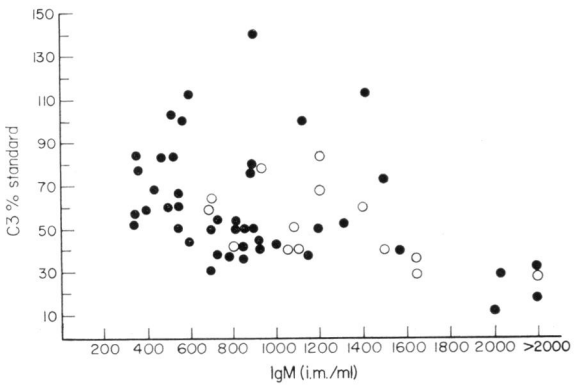

FIG. 6.

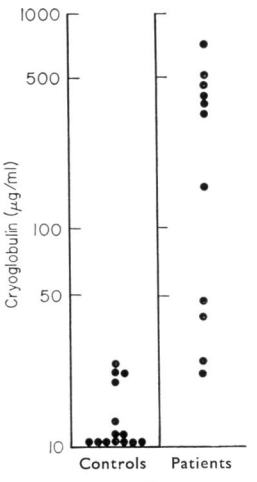

FIG. 7.

FIG. 6. Correlation between serum IgM and serum C3 levels in fifty-seven patients with Gambian sleeping sickness. (○) Patients with early disease, (●) patients with advanced disease. $r = 0{\cdot}44$.

FIG. 7. Serum cryoglobulin concentrations in eleven patients with Gambian sleeping sickness and in fourteen healthy Nigerian controls.

TABLE 1. Components of cryoglobulins detected in eleven patients with *T. b. gambiense* infection

Patient	Cryoglobulin concentration (μg/ml)	Components					
		IgA	IgG	IgM	C3	RF	TA
3	773	–	+	+	+	+	–
4	162	–	+	+	+	–	–
8	549	–	+	+	+	+	–
11	500	–	+	+	+	+	–
12	40	–	+	+	–	–	–
14	400	–	+	+	+	+	–
16	25	–	+	+	–	–	–
20	22	–	–	+	–	–	–
21	48	–	+	+	–	–	–
22	373	–	+	+	+	+	–
24	400	–	+	+	+	+	–
Total positive		0	10	11	7	6	0

RF = rheumatoid factor; TA = trypanosome antigen.

high serum IgM ($r = 0{\cdot}82$, $P = 0{\cdot}01$) and between high serum cryoglobulin concentrations and a low serum C3 ($r = 0{\cdot}56$, $P = 0{\cdot}05$).

DISCUSSION

This study has shown that most patients with Gambian sleeping sickness have low serum C3 levels which return towards normal after treatment. Both C4 and factor B levels were also depressed suggesting complement activation by both classical and alternative pathways. However a low serum factor B level cannot, by itself, be taken as evidence for complement activation by the alternative pathway as factor B may be utilized during classical pathway activation as a result of the C3b feed-back cycle (Lachmann & Nicol, 1973). Further work is required to determine whether trypanosomes or their products can activate the alternative complement pathway in a similar way to endotoxin.

Although trypanosomiasis may cause some activation of the alternative complement pathway it is likely that activation of the classical complement pathway, perhaps as a result of immune complex formation, is the major factor responsible for the low serum C3 levels found in most patients with Gambian sleeping sickness. Soluble antigens can be detected in the sera of animals infected with *T. b. gambiense* and these could be involved in immune complex formation. Patients with parasitaemia would be more likely to form complexes than patients without, perhaps explaining our finding of lower C3 levels in patients with early disease than in patients with advanced disease. Recent studies in monkeys infected with *T. b. rhodesiense* (Nagle *et al.*, 1974) and in mice infected with *T. brucei* (Lambert, 1974) have shown the occurrence of immune complex glomerulonephritis in these trypanosome infections. C3 has been detected on red cells of patients with Rhodesian sleeping sickness and this could represent complement bound to immune complexes showing immunocytoadherence (Woodruff *et al.*, 1973).

Although it is likely that immune complex formation plays an important part in producing complement activation in patients with Gambian sleeping sickness none of our patients with early or advanced disease had any clinical features of a generalized immune complex disease. None had any clinical features of glomerulonephritis but the possible presence of a mild immune complex glomerulonephritis cannot be completely

excluded as renal biopsies were not performed. Serious renal involvement has not been noted in previous studies of patients with Gambian sleeping sickness and although eye and skin lesions may occur, especially in Europeans (Apted, 1970), these are unlike the skin and eye lesions usually associated with an immune complex vasculitis. These observations suggest that if circulating immune complexes are formed in human *T. b. gambiense* infection they are not usually harmful, perhaps because they are of a sufficiently large size to be trapped and cleared rapidly by the phagocytic cells of the reticuloendothelial system. The presence of high levels of serum IgM in patients with Gambian sleeping sickness would favour the formation of large molecular weight IgM complexes that could be readily phagocytosed. High concentrations of cryoglobulins were found in the sera of most of the patients tested. The cryoglobulins contained both IgM and C3 and high concentrations were associated with a high serum IgM and a low serum C3. Formation of similar large molecular weight complexes *in vivo* could play an important part in the production of the low serum C3 levels found in most patients with sleeping sickness. Serological findings in patients with trypanosomiasis are similar to those found in patients with the tropical splenomegaly syndrome for sera of patients with this condition contain high levels of IgM, low levels of C3 and cryoglobulins containing IgM and C3 (Ziegler, 1973). Liver biopsies from patients with the tropical splenomegaly syndrome show IgM, perhaps in the form of immune complexes, within the Kupffer cells (Ziegler, 1973). It would be of interest to know whether similar deposits of IgM are present in the Kupffer cells of patients with trypanosomiasis.

In the advanced stage of *T. b. gambiense* infection trypanosomes are localized predominantly to the central nervous system and few are present in other tissues. However, patients with advanced disease still have low serum levels of C3 and C4. It is possible that at this stage of the infection immune complexes are being formed within the meninges and perivascular spaces. Activation of the complement and kinin systems (Boreham, 1970) by immune complexes formed at these sites could contribute to the meningeal and cerebral vascular damage which are important pathological features of the advanced stage of the disease (Mott, 1906). The possibility that the pathological changes of advanced Gambian sleeping sickness are due to a local Arthus reaction requires further investigation.

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