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Cryoglobulinaemia and circulating immune complexes in tropical splenomegaly syndrome

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SUMMARY

Large amounts of cryoglobulins and soluble immune complexes were detected in the sera of thirteen patients with tropical spenomegaly syndrome (TSS). Complexes were detected by three different methods: radiobioassay, a modified rheumatoid factor-binding activity method and a modified C1q-binding assay. Protein precipitable by 4% polyethylene glycol (PEG) was also measured. The cryoglobulins contained IgM, IgG and in some cases, C3. It is likely that in TSS, marked immune complex formation is associated with hypergammaglobulinaemia and that continuous engulfment of these complexes by cells of the reticuloendothelial system (RES) is the cause of the hepatosplenomegaly.

INTRODUCTION

The clinical features of tropical splenomegaly syndrome (TSS) are well-known (Lancet Leader, 1976), but the pathogenesis of the splenic enlargement characteristic of the condition remains unexplained. Ziegler (1973) postulated that TSS results from prolonged stimulation of the reticuloendothelial system (RES) by circulating macromolecular immune complexes. Indirect evidence for immune complex formation and deposition in the RES has been found. Cryoglobulins, containing IgM and IgG (and to a lesser extent IgA and C3), were found in the sera of patients with the syndrome in Uganda, and IgM (sometimes accompanied by IgG and C3) has been shown by immunofluorescence in the Kupffer cells and macrophages of the hepatic sinusoids of patients with TSS, but not in controls (Ziegler, 1973; Bryceson, Fleming & Edington, 1976). Low serum complement (C3) and anti-complementary activity have also been demonstrated in the sera of patients with the disease (Ziegler, 1973).

Immune complexes have been demonstrated on the surface of red cells in patients with some features of TSS and it has been suggested that these might play a part in the haemolytic process sometimes associated with the syndrome, as well as in the development of splenic enlargement (Woodruff *et al.*, 1973; Ssebabi *et al.*, 1975). However, there has been no previous work on measuring immune complexes in the sera of these patients. We have therefore looked for the presence of cryoglobulins and immune complexes in the sera of patients with TSS.

PATIENTS AND METHODS

Patients. Thirteen patients with untreated TSS were studied. Our criteria for making a diagnosis of TSS have been previously described (Fakunle & Greenwood, 1976). Sera from fifty healthy adult Nigerians and twenty healthy Europeans were used as controls.

Immunoglobulin measurement. Immunoglobulins were measured by the Mancini method using monospecific anti-sera (Hyland laboratories).

Preparation of cryoglobulins. 20 ml venous blood was added to a sterile container and allowed to clot at 37°C for 1 hr.

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Serum was separated and incubated at 4°C for 24 hr, by which time cryoglobulins had formed. The suspension was centrifuged at 9000 *g* at 4°C for 1 hr. The precipitates were washed three times with fresly collected distilled water at 4°C and centrifugation repeated at 9000 *g*. The final precipitate was dissolved in a small amount of phosphate-buffered saline (PBS), pH 7·2. The total protein was measured by the method of Lowry *et al.* (1951), and results expressed as μg/ml of serum. The composition of the cryoglobulins was determined by double-diffusion against monospecific antisera.

Detection of immune complexes. The following methods were used to detect immune complexes.

Radiobioassay. A radiobioassay based on competitive inhibition of uptake by aggregated γ-globulin by guinea-pig macrophages (Mohammed, Thompson & Holborow, 1977) was used.

Rheumatoid factor-binding assay. A modified rheumatoid factor (RF) binding method was used (Cowdery, Treadwell & Fritz, 1975). RF was isolated from 10 ml serum from a patient with a strongly positive DAT test using Degalan beads coated with aggregated human IgG as an immunoabsorbent. RF was eluated at pH 2·5. The eluant was shown to possess RF activity using sensitized latex particles. It was purified by passing through a column of Sephadex G-200, and then concentrated with Lyphogel.

C1q-binding. Immune complexes were detected by an ^{125}I-labelled C1q assay as previously described (Zubler *et al.*, 1976).

Polyethylene glycol method (PEG). Sera were diluted 1:5 and mixed with equal amounts of 8% PEG to reach a final concentration of 4%. The precipitates formed were washed, and their total protein measured as described by Lowry *et al.* (1951).

Detection of immunoglobulin in circulating polymorphoneutrophil leucocytes. Blood was sedimented with dextran, the supernatant removed and centrifuged and the cell button washed three times in tissue culture medium. Thin smears were made, fixed in methanol and stained for IgM, IgG and C3 by direct immunofluorescence.

Fluorescence in the polymorphoneutrophil leucocytes was scored 3+ if many polymorphoneutrophil leucocytes stained brightly, 2+ if few stained brightly, + if only slight staining was noted, or − if no staining was detected.

In a separate experiment, polymorphoneutrophil leucocytes from healthy donors were incubated *in vitro* in pooled TSS and control sera for 30 min, and then stained for intracytoplasmic inclusions as above.

RESULTS

Table 1 shows the results of IgM, cryoglobulin and immune complex determinations in thirteen patients with TSS. Patients had significantly higher levels of cryoglobulins and circulating immune complexes than controls. Cryoglobulins contained IgM and IgG in eleven instances, and IgM alone in two instances.

TABLE 1. Splenic size, IgM, cryoglobulin and immune complexes in patients with TSS and controls

Patients (number)	Spleen size (cm)	IgM (iu/ml)	Cryoglobulin (μg/ml)	Radiobioassay (per cent inhibition)	RF-binding assay (per cent inhibition)	C1q (per cent binding activity)	Precipitation with 4% PEG (g/l)
1	15	4200	580	58	46	—	—
2	21	4620	540	54	40	—	—
3	8	7600	1400	64	50	—	—
4	19	4600	340	50	42	—	—
5	18	3040	1630	30	24	87	—
6	13	1376	1600	30	24	77	3·60
7	10	1337	235	41	30	60	2·70
8	16	6000	1630	62	48	62	2·10
9	35	2432	400	44	35	95	5·02
10	20	1000	390	38	28	94	3·65
11	11	5010	700	34	28	56	2·17
12	10	1352	1600	35	29	35	0·45
13	25	3800	610	50	39	98	4·00
Mean ± s.d.	17 ± 7·4	3566·7 ± 2035·7	896·5 ± 571·4	45·4 ± 11·8	35·6 ± 9·1	73·8 ± 21·7	2·96 ± 0·75
Control mean ± s.d.	0	261 ± 136·5 (n = 50)	26·5 ± 15 (n = 15)	22·5 ± 2·9 (n = 45)	15·7 ± 2·1 (n = 45)	22·9 ± 11·4 (n = 10)	0 (n = 20)

Using Students' t-test of significance of difference between means, $P < 0·0005$ in each instance.

TABLE 2. IgG, IgM, and C3 inclusions in TSS and control leucocytes detected by direct immunofluorescent staining

Patients (number)	IgG	IgM	C3
1	−	—	−
2	+	2+	−
3	+	3+	−
4	−	+	−
5	+	3+	−
6	−	2+	−
7	+	+	+
8	+	2+	−
9	−	+	−
10	+	3+	+
Controls (n = 10)	−	−	−

C3 was detected in only two precipitates. Analysis of the protein precipitated with PEG showed that the mean IgM content was 54 iu/ml and the mean IgG content was 3·4 iu/ml.

Table 2 shows the results of immunofluorescent staining of polymorphoneutrophil leucocytes. Immunoglobulin inclusions were seen in nine out of ten patients with TSS, but in none of the controls. An anti-IgM conjugate gave the brightest staining. On incubation with TSS serum, normal polymorphoneutrophil leucocytes ingested large amounts of immunoglobulin, especially IgM. Only occasional inclusions were seen on incubation of polymorphoneutrophil leucocytes with normal Nigerian sera.

A significant correlation was found between serum IgM and serum immune complex concentrations as determined by the radiobioassay and rheumatoid factor-binding tests ($r = 0{\cdot}86$, $P<0{\cdot}001$; $r = 0{\cdot}78$, $P<0{\cdot}001$, respectively). Fig. 1 shows a remarkable correlation between percentage inhibition by the

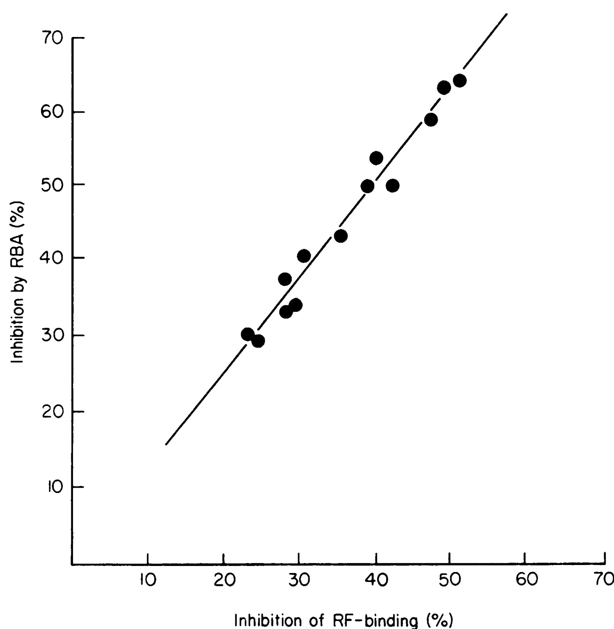

FIG. 1. The correlation between the percentage inhibition by the RBA and the percentage inhibition of the rheumatoid factor-binding assay.

radiobioassay (RBA) and percentage inhibition by the rheumatoid factor-binding assay, indicating a degree of homogeneity in the complexes found in TSS. However, there was no significant correlation between IgM and cryoglobulin levels, or between circulating immune complex concentrations and splenic size.

DISCUSSION

This study shows that sera of patients with the tropical splenomegaly syndrome (TSS) contain high levels of cryoglobulins and immune complexes. The results of PEG precipitation and of incubating TSS sera with polymorphoneutrophil leucocytes show that these immune complexes contain large amounts of IgM.

In a previous study, it proved impossible to demonstrate malarial antigen in the cryoglobulins (Ziegler, 1973), and it remains uncertain whether the complexes found in TSS are in fact malarial antigen–antibody complexes.

It is possible that when serum IgM is very high, non-specific aggregation occurs *in vivo* analogous to cryoglobulin formation *in vitro*. This view is supported by our finding of a correlation between serum IgM and immune complex levels. Complexes which contain large amounts of IgM are likely to be of very high molecular weight and may therefore be preferentially phagocytosed by cells of the reticulo-endothelial system (RES), rather than be deposited in the kidneys or small blood vessels. In fact, these patients do not have nephritis, cutaneous vasculitis or other evidence of immunopathology arising from extracellular deposition of immune complexes. They appear to be forming excessive amounts of large-sized immune complexes which present a persistent challenge to the phagocytic potential of the RES. If this is so, it seems likely that in such patients plasmaphoresis might be beneficial. No correlation was found between immune complex level and splenic size, but the former is likely to fluctuate considerably from time to time and clinical assessment is a poor indicator of splenic size.

It is likely that hypergammaglobulinaemia and immune complex formation are the major abnormalities in TSS and precede the clinical features of the syndrome. The pathogenesis of this hypergammaglobulinaemia, which is in some way related to repeated malarial infection, is unknown, but a defect in the normal control mechanisms governing immunoglobulin synthesis seems a likely possibility (Fakunle & Greenwood, 1976).

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