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CRYOGLOBULINS IN VASCULITIS

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SUMMARY

Serum cryoglobulin levels were estimated in thirty-one patients with cutaneous vasculitis, twenty-nine patients with other skin disorders and forty-seven normal subjects. Eighteen of the normal sera contained mixed cryoglobulins—the upper limit of the normal range being 80 μg/ml. Twelve of the vasculitis patients had serum cryoglobulin levels above the normal range. All but one of these cryoglobulins were mixed cryoglobulins. A low serum C3 level was found in only one patient. The possible role of mixed cryoglobulins in the pathogenesis of vasculitis is discussed.

INTRODUCTION

Cutaneous vasculitis, for which the terms Schönlein-Henoch purpura, anaphylactoid purpura and allergic vasculitis are used, is characterized by crops of urticaria, erythema and purpura. Arthritis, intestinal and renal disease may also occur.

A number of models of vasculitis have been studied in experimental animals including experimental serum sickness (Dixon *et al.*, 1958), generalized and local Shwartzman reactions and the model described by Selye & Tuchweber (1965) in which haemorrhagic lesions follow the injection of agar intravenously and adrenaline subcutaneously. The Shwartzman reactions and the model of Selye & Tuchweber would appear to correspond in man to the disorders seen in profoundly ill patients suffering from Gram-negative septicaemia in pregnancy or fulminant meningococcaemia, and clinically are quite different from Schönlein-Henoch purpura (Hjort & Rapaport, 1965; Meyers, Hirschman & Sloan, 1970). 'One-shot' serum sickness would appear to be the animal model which most closely resembles the Schönlein-Henoch syndrome in man. Widespread vascular lesions histologically similar to the Arthus phenomenon develop after the administration of a single dose of antigen. The components of antigen–antibody complexes have been detected in the vessels of patients with cutaneous vasculitis by several workers (Stringa *et al.*, 1967; Parish, 1971). Techniques for detecting immune complexes in the serum have been developed and these include

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analytical ultracentrifugation, examination of the pellet obtained after ultracentrifugation of serum (Almeida & Waterson, 1969; Gocke *et al.*, 1970) and identification of complement in macromolecular fractions (Soothill & Hendrickse, 1967). Mixed cryoglobulinaemia also furnishes evidence that antigen is combining with antibody in serum since such cryoglobulins are made up of two different immunoglobulins, one of which can be shown to be an antibody against the other.

Mixed cryoglobulinaemia has been sought in patients with cutaneous vasculitis and the results are reported here.

PATIENTS AND METHODS

Thirty-one patients with cutaneous vasculitis were studied. All had normal platelet counts. Twenty-nine had crops of purpura of the type seen in Schönlein–Henoch purpura (Cream, Gumpel & Peachey, 1970) and two of these patients had additional clinical features which in one enabled the diagnosis of systemic periarteritis nodosa to be made whilst the other had plaques typical of erythema elevatum diutinum. Two other patients who did not have crops of purpura, one with erythema elevatum diutinum and one with Degos' malignant atrophic papulosis are included. The clinical features of the patients will be reported in full elsewhere.

Five patients had renal involvement and one of these had positive blood cultures (*Staph. albus*) but also later developed LE cells and a positive ANF test. One other patient had a positive ANF test but no LE cells.

Forty-seven normal subjects and thirty-two patients with miscellaneous skin disorders served as controls.

Serum cryoglobulins

50 ml of blood, taken into pre-warmed syringes and bottles, was allowed to stand for at least 4 hr at 37°C before being spun twice at 1750 ***g*** for 10 min at 37°C. The separated serum was stored for 7 days at 4°C and then spun at 22,000 ***g*** in an ultracentrifuge for 30 min at 4°C. The precipitate was washed four times in cold PBS. It was redissolved in a known volume of PBS, usually 1 ml, at 37°C for 1 hr and then any insoluble material was removed by centrifugation at 1750 ***g*** for 10 min at 37°C immediately prior to further analysis.

The total protein content of the cryoglobulin solution was determined by the method of Lowry *et al.* (1951) and immunoelectrophoresis was carried out using rabbit anti-whole human serum prepared in this laboratory, and rabbit anti-human heavy chain specific sera—anti-α, anti-γ and anti-μ, obtained from Hoechst. Proteins not detected by immunoelectrophoresis were further sought by double diffusion in agar as were C3, albumin and α_2-macroglobulin using Hoechst antisera.

Detection of rheumatoid factor activity in cryoglobulin

An FII slide latex test (Hyland) was carried out on warmed glass slides.

Serum immunoglobulins and C3 were estimated in serum separated at 37°C using Hyland 'Immunoplates' incubated at 37°C for 8 hr.

Bence-Jones proteinuria. Urine was concentrated forty times by freeze-drying and immunoelectrophoresed against anti-whole human serum. Free light chains were distinguished

by their failure to react with heavy chain specific antisera and were identified using anti-κ and anti-λ sera.

RESULTS

Serum immunoglobulins

The log 2SD ranges in the normal subjects were IgG 935–2964 mg/100 ml, IgA 93–677 mg/100 ml and IgM 51–298 mg/100 ml. Ten of the patients with vasculitis had abnormal immunoglobulin levels (Table 1).

TABLE 1. Patients with abnormal serum immunoglobulin levels

Patient	Duration of disease	Cryoglobulin		Serum immunoglobulin (mg/100 ml)		
		μg/ml serum (Folin)	Components	IgG	IgA	IgM
Normal	—	<80		935–2964	93–677	51–298
Gre.	2 years	1250	IgG	3770	165	45
Llo.	6 years	375	IgG–IgA	1150	1120	115
Wol.	6 years	340	IgG–IgM	2300	500	800
Alv.	3 years	395	IgG–IgA	1900	1000	80
Gol.	2 years	85	IgG–IgA	1120	160	15
Del.	2 weeks	85	IgG–IgA	3200	340	140
Har.	3 weeks	26	—	1100	245	20
Rob.	6 months	10	—	2600	340	10
Kni.	20 years	30	—	1250	85	110
Hut.	20 years	51	—	1520	180	30

Cryoglobulins

Serum cryoglobulin levels in the normal subjects, the patients with miscellaneous skin disorders and those with vasculitis are shown in Fig. 1. The upper limit of the log 2SD range for the normal group was 80 μg/ml. The levels found in the thirty-two patients with miscellaneous skin disorders were similar but one patient with psoriasis had a serum cryoglobulin of 190 μg/ml for which there was no obvious explanation. Twelve patients with vasculitis had high serum cryoglobulin levels.

In one patient (Gre., with a monoclonal IgG) cryoprecipitin occurred rapidly and the serum became cloudy within seconds of its removal from the 37°C water bath. In most of the others a sediment had formed at the bottom of the tube after standing overnight at 4°C. In two, however (Llo., Alv.) both with IgG–IgA cryoglobulins, although the serum had become cloudy and cleared on warming, no sedimentation was apparent even after 7 days, whilst in two other sera neither sediment not turbidity appeared until after 3 or 4 days. In the sera containing mixed cryoglobulins precipitation continued in the supernatant obtained after centrifugation at 7 days.

On immunoelectrophoresis of the cryoglobulins against anti-whole human serum only

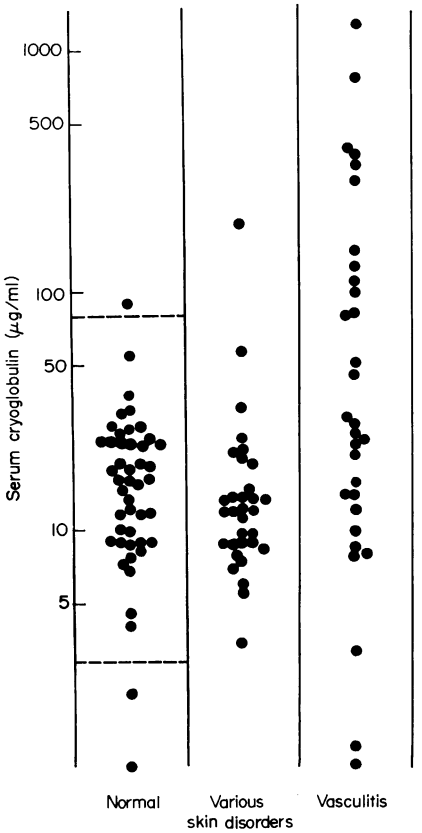

FIG. 1. Serum cryoglobulin levels in normal subjects, patients with various skin disorders and patients with vasculitis.

immunoglobulin lines developed. On double diffusion in agar C3 was detected in four and albumin in two of the preparations, both of these being IgG–IgA complexes. α_2-macroglobulin was not detected in any.

The results of the analyses for immunoglobulins, complement and rheumatoid factor activity in the cryoglobulins for the normal subjects and the vasculitis patients are shown in Table 2.

Serum C3 (β_1C/β_1A globulin)

The results for the normal group, the patients with normal levels of cryoglobulin and those with raised levels are shown in Fig. 2. The log 2SD range for the normal group was 91·7–239 mg/100 ml. Two patients had C3 levels outside this range. One with a C3 of 45 mg/100 ml had a mixed IgG–IgM cryoglobulin and persistent microscopic haematuria. The other with a C3 of 360 mg/100 ml had microscopic haematuria but no cryglobulinaemia. The geometric mean for the mixed cryoglobulinaemic group was lower than that for the normal group but this was only just significant at the 5% level ($t=1{\cdot}77$, $0{\cdot}05>P>0{\cdot}025$). The non-cryoglobulinaemic vasculitis group had a significantly higher geometric mean than the normal group ($t=2{\cdot}13$, $0{\cdot}025>P>0{\cdot}0125$). The C3 levels in the patients with single component cryoglobulinaemia, periarteritis nodosa, erythema elevatum diutinum and Degos'

TABLE 2. Results of analyses of cryoprecipitates from normal subjects and patients with vasculitis

	No. of subjects	Cryoprecipitate: Immunoglobulins detected	Cryoprecipitate: No. in which C3 detected	Cryoprecipitate: No. with rheumatoid factor activity
A. Normal subjects	13	None	0	0
	2	IgG	0	0
	4	IgM	0	0
	25	IgG–IgM	0	16
	2	IgG–IgM–IgA	0	2
B. Vasculitis, normal serum cryoglobulin	8	None	0	0
	2	IgM	0	2
	9	IgG–IgM	0	6
C. Vasculitis, raised serum cryoglobulin	1	IgG (γ, κ)	0	0
	5	IgG–IgM*	2	5
	5	IgG–IgA†	2	5
	1	IgG–IgM–IgA	0	1

* Traces of IgA detectable in four.
† Traces of IgM detectable in four.

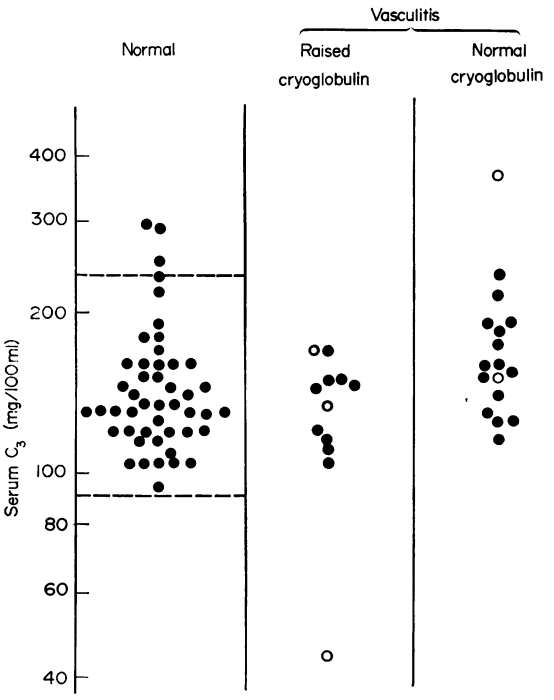

FIG. 2. Serum C3 levels in normal subjects, vasculitis patients with raised serum cryoglobulin levels and normal cryoglobulin levels. ○, Renal disease; ●, no renal disease.

malignant atrophic papulosis were within the normal range. The serum C3 levels in the patients who had mixed cryoglobulins containing complement were 170, 150, 145 and 45 mg/100 ml.

Bone marrow skeletal surveys and Bence-Jones proteinuria

Nine of the patients who had raised serum cryoglobulin levels had bone marrow biopsies, skeletal surveys and the urine was examined for Bence-Jones protein. None had an abnormal marrow or skeletal survey. Kappa chains were found in the urine of one patient (Ma.) who had an IgG–IgM cryoglobulin with a kappa IgM component.

DISCUSSION

In this study, mixed cryoglobulins were found in low concentration in some normal sera and in much higher concentration in the sera from some patients with vasculitis. In such cryoglobulins it has been shown that the IgM or IgA component is an antiglobulin against the IgG component (Lo Spolluto *et al.*, 1962; Matuhasi, Usui & Mizuno, 1968).

In normal sera rheumatoid factors are commonly present in low titre (Ball & Lawrence, 1961). The demonstration of mixed cryoglobulins in normal subjects in this study provides confirmation that they too may produce small amounts of antiglobulin capable of reacting with their own IgG. The serum cryoglobulin level was not related to the age in the normal subjects (Fig. 3). (Correlation coefficient, $r=0{\cdot}2782$, $t=1{\cdot}92$, $P>0{\cdot}05$.)

Vasculitis occurring in crops may present a clinical picture varying from a trivial rash to

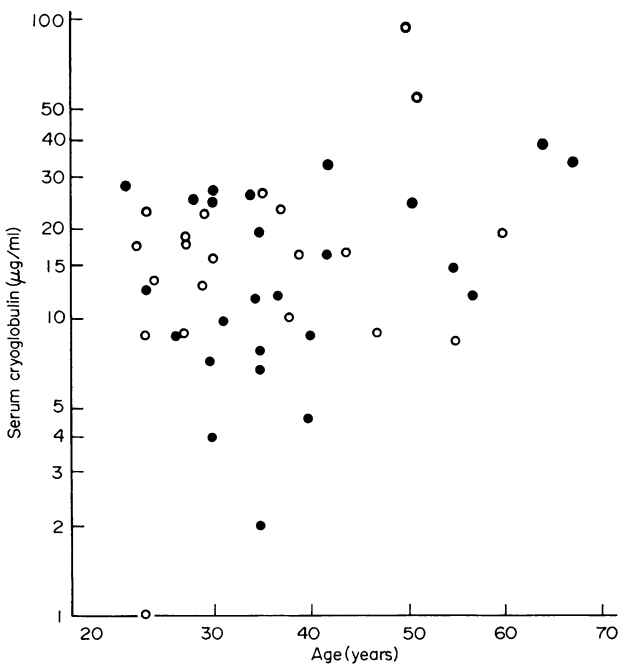

FIG. 3. Serum cryoglobulin levels and age in normal subjects. ○, Males; ●, females.

a syndrome in which purpura is accompanied by arthritis, gastro-intestinal and renal involvement. Amonst the known causes of this clinical picture are serum sickness, sub-acute bacterial endocarditis, systemic lupus erythematosus and chronic meningococcaemia. All too often no cause is found and use of the descriptive terms Schönlein–Henoch purpura, anaphylactoid purpura, or allergic vasculitis is then expedient.

The pattern of disease, a widespread multifocal vasculitis, a histology similar to that seen in the Arthus phenomenon and the frequency of preceding infections (Bywaters, Isdale & Kempton, 1957; Cream *et al.*, 1970) suggest that circulating immune complexes may be involved. Immunoglobulin, complement and antigens from infectious agents have been detected in the damaged vessels (Stringa *et al.*, 1967; Parish, 1971).

The finding of mixed cryoglobulins in serum has been taken as evidence of circulating immune complex disease (Barnett *et al.*, 1970) so that mixed cryoglobulinaemia in approximately one third of patients with cutaneous vasculitis would appear to provide direct evidence of circulating immune complexes in these patients.

Mixed cryoglobulinaemia has been previously noted in patients with glomerulonephritis and cutaneous vasculitis. The possibility that these cold precipitable complexes circulate as immune complexes in serum is supported by reports that immunoglobulins of the same classes as those in the cryoglobulins may be detectable in the damaged glomeruli (Loghem–Langereis *et al.*, 1965; Meltzer *et al.*, 1966; Golde & Epstein, 1968; Feizi & Gitlin, 1969; McIntosh *et al.*, 1970) and also in the skin lesions (Miescher, Paronetto & Koffler, 1965; Douglas, Lahav & Fudenberg, 1970; Cream, 1971). The pathological properties of these cryoglobulins has been demonstrated by McIntosh, Kulvinskas & Kaufman (1971) who observed erythema and haemorrhage after intradermal injection into guinea-pigs and glomerulitis after intravenous infusion.

An unexpected finding in these patients was that the high levels of cryoglobulin were found predominantly in those who had had vasculitis for more than 1 year whilst in those whose disease had been active for a shorter period serum cryoglobulins were either normal or only slightly increased (Fig. 4). This suggests that mixed cryoglobulinaemia may be an entirely secondary phenomenon or merely one of several factors necessary for the development of vasculitis.

Hypocomplementaemia has been shown to occur in experimental serum sickness (Weigle & Dixon, 1958) and in what are believed to be circulating immune complex diseases in man—post-streptococcal nephritis and systemic lupus erythematosus (Derrick, Reeves & Dillon, 1970; Stastny & Ziff, 1969; Townes, Stewart & Osler, 1963) but is not invariable and in serum sickness in man the complement level may be normal (Vaughan, Barnett & Leadley, 1967).

Serum complement, either CH_{50} or C3, has been previously studied in anaphylactoid purpura or cutaneous vasculitis. Of a combined total of ninety-five patients, at least sixty-six of whom had nephritis, hypocomplementaemia was found in ten (Michael *et al.*, 1967; Gewurz *et al.*, 1968; McDuffie, 1970) whilst normal and raised levels were encountered in the majority (Wedgewood & Janeway, 1953; Williams & Law, 1958; West, Northway & David, 1964; Gotoff *et al.*, 1965; Ogg, Cameron & White, 1968; Kohler & ten Bensel, 1969; Ayoub & Hoyer, 1969). The association of hypocomplementaemia and mixed cryoglobulinaemia has been noted in post-streptococcal nephritis (McIntosh *et al.*, 1970) in purpura (Riethmuller *et al.*, 1966; Douglas *et al.*, 1970) and in various other disorders (Bonomo *et al.*, 1970). In the present series a serum C3 level below the normal range was

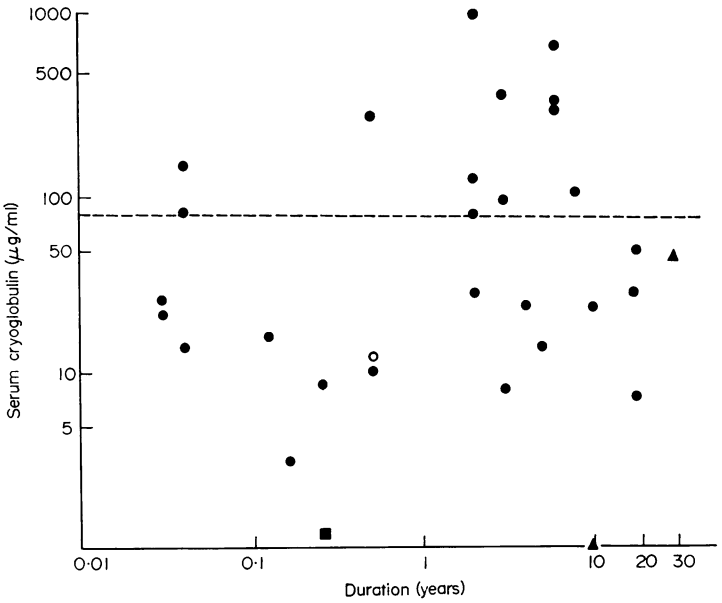

FIG. 4. Vasculitis patients—serum cryoglobulin levels and duration of disease. ■, Periarteritis nodosa; ▲, erythema elevatum diutinum; ○, Degos' syndrome.

found in only one patient who had renal disease and a high serum cryoglobulin level. With the exception of this patient the serum complement results failed to provide convincing evidence that would support a circulating immune complex pathogenesis.

Classifications of cutaneous vasculitis, at present based on variable clinical and histological appearances, are unsatisfactory and have led to the use of many confusing terms (Winkelmann & Ditto, 1964). Whatever the precise role of the cryoglobulin, it would appear from this study that patients who have crops of purpura can be simply divided into two groups (1) non-cryoglobulinaemic vasculitis and (2) cryoglobulinaemic vasculitis with either (a) a single component cryoglobulin or more commonly (b) an immune complex cryoglobulin.

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