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SERUM COMPLEMENT LEVELS IN PATIENTS WITH MIXED (IgM–IgG) CRYOGLOBULINAEMIA

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SUMMARY

Serum complement levels were determined in seven patients with mixed (IgM–IgG) cryoglobulinaemia. All seven patients had either no measurable or very low levels of C'2. C'50 haemolytic units were determined in three patients and were also found low, although less striking. The mechanism by which the blood of these patients became depleted of C'2 could not be determined.

One of the more common types of cryoglobulins, which was initially described by LoSpalluto *et al.* (1962), consists of a mixture of the two immunoglobulins IgM and IgG. Because cryoprecipitation requires the presence of IgM from the patient and IgG from any source, these proteins might best be called 'mixed cryoglobulins' (Meltzer & Franklin, 1962; Meltzer *et al.*, 1966). In contrast, the more widely recognized cryoglobulins, which are generally seen in multiple myeloma or macroglobulinaemia, consist either of IgM or IgG molecules. Since the mixed cryoglobulins are always associated with rheumatoid factor activity and since their precipitation has a superficial resemblance to antigen–antibody reactions, it appeared of interest to investigate the possible role of complement in the 'mixed' cryoglobulin syndrome. For this purpose, complement levels were determined in seven patients suffering from recurrent vascular purpura and arthralgia and exhibiting the IgM–IgG type of cryoglobulin.

In their clinical aspects, this group of patients appeared to be rather homogeneous. All but one of eleven patients were females; their ages at the onset of their illness ranged from 20 to 50 years. Minor lymphadenopathy was a common physical finding and six patients had either mild hepatomegaly or splenomegaly. All of the eleven patients gave a similar history of arthralgias, Raynaud's phenomenon and recurrent purpuric eruptions, especially of the lower extremities. Three patients died from a rapidly progressive renal failure with evidence of γ-globulin deposition in renal glomeruli. The other patients had no evidence of renal involvement. The detailed clinical and laboratory features of these patients will be presented separately (Meltzer *et al.*, 1966; Miescher, Koffler & Paronetto, 1966).

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Because of the suggestion (Austen & Beer, 1964) that the determination of C'2 is a more sensitive assay for *in vivo* complement consumption than the determination of complement in terms of haemolytic units, both total haemolytic complement and C'2 levels were determined in seven of these patients.

MATERIALS AND METHODS

Fresh sheep cells, delivered in Alsevers solution, and Amboceptor were supplied by Baltimore Biological Laboratories. Lyophilized pooled guinea-pig serum from Certified Blood Donors served as source for the complement components C'1, C'4 and C'3. Serum was generally obtained from freshly drawn blood and assayed immediately for complement level. The sera from two patients F.C. and M.Q. who had died were used after storage below

TABLE 1

Patients	C'2 reciprocal of titre	C'50
B.S.	0	9·2
G.D.	0	16·2
F.M.	0	4·8
M.Q.	0	n.d.
F.C.	220	n.d.
G.K.	0	n.d.
V.D.	60	n.d.

	C'2		C'50	
	Range	Mean	Range	Mean
Normals (12)	350–650	475	25–40	33·8
SLE (5)	82–380	215	—	—

−20°C for 2 years and several weeks, respectively. C'2 was determined by the method of Austen & Beer, 1964, by its specific interaction with a cellular intermediate prepared with guinea-pig complement, the EAC'$1a_{gp}$,4_{gp} cell. Total haemolytic serum complement was measured after Mayer as described in Kabat & Mayer (1961).

The cryoglobulins were isolated at 0°C, washed repeatedly with 0·15 M saline and shown to consist only of IgM and IgG molecules by analytic ultracentrifugation and by immunologic analysis with antisera to the various immunoglobulins and to whole normal human serum (Meltzer *et al.*, 1966).

RESULTS

Six patients had C'2 levels which were below the sensitivity level of the methods (0–60 units) and one patient had a level of 220 units (Table 1). The total complement levels were determined in three patients. They were also distinctly reduced, although not to the same degree

as the C'2 levels. Control determinations in normal subjects ranged from 350 to 650 units for C'2 and from 25 to 40 units for total C'. Table 1 shows that somewhat reduced levels were also found in five patients with systemic lupus erythematosus, but not in two other subjects with purpura unassociated with mixed cryoglobulins.

DISCUSSION

The observation of consistently low C'2 levels in seven subjects with mixed cryoglobulins all of which possess rheumatoid-like factor anti-γ-globulin activity, suggests that complement may play a role in the pathogenesis of some of the lesions of this disorder. One might expect that the IgM–IgG interaction fixes complement. *In vitro* studies failed to provide such a simple explanation for the low serum levels of C'2. When this cryoprecipitate was formed in normal serum, by adding either patient serum or cryoprecipitate which has been dissolved in saline, no complement was fixed as measured by C'2 determinations or by assaying the haemolytic complement activity of the serum. This is similar to the lack of complement fixation by the interaction of classical 'warm rheumatoid factors' and γ-globulin. Furthermore, it is interesting to note that immunoelectrophoretic examination of fresh serum from the patients with almost no C'2 activity revealed a β-1C precipitation line which was not different from that of normal fresh serum. Thus the precise mechanism by which the blood of these patients gets depleted of C'2 could not be determined, and additional studies are obviously required.

Regardless of its basic significance, the finding of low C'2 levels in certain subjects with unusual clinical features should suggest the possible presence of mixed cryoglobulins and, indeed, in one of the subjects of this study, search for cryoglobulins was made only after the discovery of the low C'2 level.

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