

IMMUNOBIOLOGICAL ASPECTS OF Clq IN SERA OF PATIENTS WITH CUTANEOUS VASCULITIS AND COLLAGEN DISEASES

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Abstract. Clq was comparatively quantified with CH50 or C3 in sera of patients with various types of cutaneous vasculitis and collagen diseases. The following results were found: 1) Elevated levels of Clq were seen much more frequently in cutaneous vasculitis and PSS. 2) No significant correlations were found between Clq and CH50 or C3, except for a moderate rank correlation between Clq and C3 in SLE. 3) The amount of hydroxyproline in serum (collagen-like protein) is nearly identical with the calculated value of that present in Clq.

Key words: Clq; Collagen diseases; Cutaneous vasculitis

Some forms of vasculitis and collagen diseases are considered to be cutaneous expressions of immune complex diseases. As a matter of fact, immunofluorescent studies have already demonstrated deposition of Clq and C3 as well as immunoglobulin lining vessel walls in cutaneous vasculitis (19, 20), and that lining the dermal-epidermal junction of skin lesions in systemic lupus erythematosus (SLE) (5). These findings were consistent with the observation of a low complement (C) level in sera from patients with SLE and other collagen-vascular diseases (12, 21, 22) and less often in sera from progressive systemic sclerosis (PSS) (25).

The C system consists of a set of 12 distinct proteins, forming a complex biological system that fulfils various functions. This system is not only important in host defence mechanisms but also takes part in disease processes involving cytotoxic and immune-complex mediated hypersensitivities. Clq is the triggering component in the classical train of the C system and can bind not only to antigen-antibody complexes but presumably non-specifically to denatured, soluble, altered immunoglobulins and other polyanionic substances (1), subsequently activating succeeding C compo-

nents. Interestingly, Clq contains hydroxyproline, hydroxylysine and abundant glycine residues in its molecule. Analyses of its carbohydrates have revealed that they are galactosylglucosyl residues coupled to hydroxylysine, which are similar to those of glomerular basal membrane collagen (23). Its hemolytic activity is easily destroyed by bacterial collagenase on digestion (15) and recent extensive chemical studies have disclosed that Clq is an unusual globular protein and that about a half of the Clq molecule is composed of a triple helix with collagen-like sequence (16). Furthermore, the production and secretion of Clq by fibroblasts (2, 17) and a competitive inhibition of collagen-induced platelet aggregation by Clq (4, 24, 26) has been reported.

On the other hand, LeRoy et al. (8) stated that bound hydroxyproline (so-called collagen-like protein) was present in plasma and originated from or was otherwise related to collagen. Although the fluctuation of concentration of collagen-like protein (CLP) in sera from patients with connective tissue diseases has been reported previously (6), very little information was available on possible relations between CLP and collagen. The present study was therefore undertaken to quantify serum Clq levels in comparison with total hemolytic C activity and with C3, the essential C component in the alternate pathway, ultimately in pursuit of any possible relations between Clq and CLP in sera of patients with cutaneous vasculitis and collagen diseases.

MATERIALS AND METHODS

Patient population

The subjects who participated in this study were chosen from among patients being cared for at our hospital. Those with uncertain diagnoses were excluded from the study.

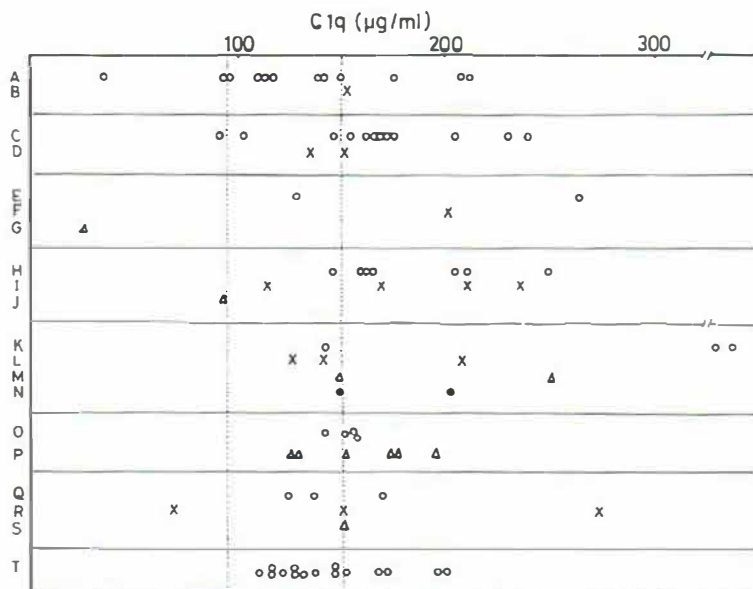


Fig. 1. C1q level in 85 patients suffering from various skin diseases. A, systemic lupus erythematosus; B, discoid lupus erythematosus; C, progressive systemic sclerosis; D, morphea; E, dermatomyositis; F, periarteritis nodosa; G, mixed connective tissue disease; H, Behçet disease; I, pyoderma gangrenosum; J, Wegener granulomatosis; K, anaphylactoid purpura; L, livedo angitis; M, thrombophlebitis; N, Mucha-Habermann disease; O, erythema nodosum; P, erythema induratum Bazin; Q, pemphigus vulgaris; R, bullous pemphigoid; S, subcorneal pustular dermatosis; T, other skin diseases. ---, normal range.

and in consequence the total number of patients examined was 85. The study population consisted of patients suffering from lupus erythematosus (13 cases), scleroderma (14 cases), dermatomyositis and other collagen diseases (4 cases), diverse skin diseases comprising various types of angitis (32 cases), bullous diseases (7 cases) and miscellaneous (15 cases).

The blood samples collected by venepuncture were allowed to clot at room temperature for 60 min and then to contract at 4°C for 120 min. Serum was separated and stored in small aliquots at -70°C until studied.

Assay of C activities

Total hemolytic complement (CH50) determination was performed according to the method of Mayer (11). The normal range for the serum of an adult Japanese is from 30 to 50 CH50 units/ml.

Immunochemical quantitation of C1q and C3

This was carried out by means of single radial immunodiffusion (SRID) according to a modification of the method of Mancini et al. (10). Anti-human C1q or anti-human C3 serum was prepared in accordance with Yonemasu's method (28) or by the method described earlier (14). The normal ranges of these in Japanese adults are 136.5 ± 25.0 µg/ml for C1q, 1108 ± 257 µg/ml for C3 (29).

Determination of hydroxyproline

This was conducted spectrophotometrically by a method described previously (7). The hydroxyproline possessed by C1q as a molecular constituent was calculated from the C1q amount measured by SRID, using percentage weight of hydroxyproline to C1q obtained from a chemical study of C1q as outlined elsewhere (27).

RESULTS

1. C1q level

Serum concentrations of C1q were determined in 85 patients with a wide variety of skin diseases. The results are shown in Fig. 1 and are classified by sort and/or type of disease.

Eight of 12 patients with SLE, who were in the non-active stage of the disease, showed normal C1q concentrations, 3 the higher, and 1 the lower. In 9 of 12 patients with PSS, the level of C1q was elevated and in the remainder it was still in the normal range. Although the number of patients involved was small, it may be stated that 1 patient with DLE and 2 with morphea had a normal level of C1q. In 1 of 2 patients with dermatomyositis and 1 with periarteritis nodosa, the C1q level was elevated. A marked fall in the level of C1q was noted in 1 case diagnosed as mixed connective tissue disease.

In most of the patients who had skin eruptions in the active stage of Behçet disease or pyoderma gangrenosum, C1q was augmented remarkably. Of the patients with different types of cutaneous vasculitis, 2 with anaphylactoid purpura in the active stage presented the conspicuously high C1q values of 560 and 330 µg/ml, respectively, and 1 with chronic capillaritis had a normal value. In one patient with livedo angitis who complained of joint pain and one with thrombophlebitis, the C1q level was found to be elevated. A greater-than-normal

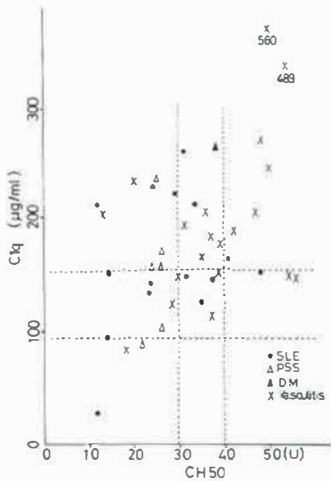


Fig. 2. Clq level and total hemolytic complement. ● SLE, systemic lupus erythematosus; △ PSS, progressive systemic sclerosis; ▲ DM, dermatomyositis, × vasculitis, including various types. ---, normal range.

Clq value was noted in the active stage of Mucha-Habermann disease, while it was normal or else slightly elevated in erythema nodosum and erythema induratum Bazin.

Among bullous dermatoses, 1 case of bullous pemphigoid showed an extremely high level of Clq, the rest (3 of pemphigus vulgaris and 2 of bullous pemphigoid) giving normal concentrations. All the 15 patients in the miscellaneous category showed Clq values within the normal limits, except the two, one suffering from pustulosis palmaris et plantaris

and the other from drug eruption (erythroderma), who yielded above normal values.

2. Clq level and total hemolytic complement (CH50)

Both Clq and CH50 in 44 patients were quantified and plotted in a scatter-diagram (Fig. 2). Although the number of patients examined was not large enough to warrant mention of anything definite concerning correlations between the two, the figure shows that in all cases of PSS and in a majority of SLE cases the level of CH50 was found to be lower, regardless of the level of Clq. In nearly all cases of cutaneous vasculitis, Clq values were above the normal range.

3. Clq and C3 levels

In 70 patients, Clq and C3 were quantified and both were plotted in a scatter-diagram. No significant correlations between Clq and C3 were seen in any of the skin diseases concerned. As shown in Fig. 3, only in SLE was a moderate degree of positive correlation found, with 0.544 of Kendall's rank correlation coefficient, which was significant at the 0.01 % level. In PSS, Clq was in or above the normal range. A majority of cutaneous vasculitis cases including Behçet disease and pyoderma gangrenosum showed high levels of Clq. C3 concentrations were mostly normal in these cutaneous disorders but lower in some exceptional cases.

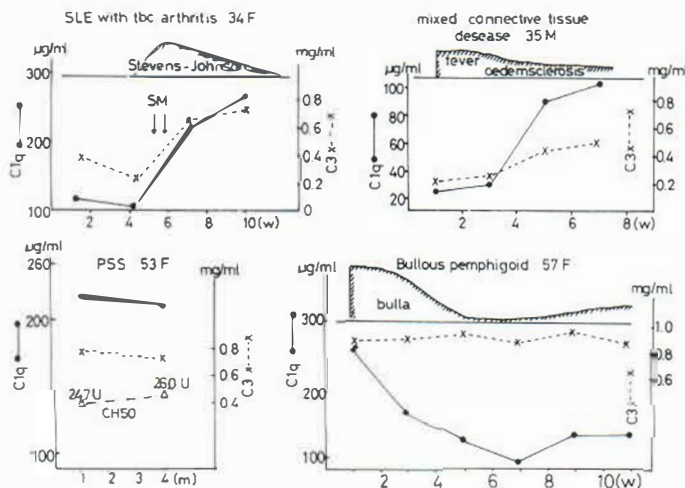


Fig. 3. Clq, C3 level and clinical course.

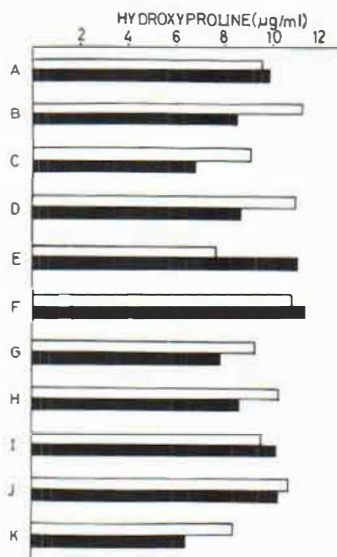


Fig. 4. Total hydroxyproline in sera and that derived from Clq. □, serum hydroxyproline; ■, theoretical hydroxyproline from Clq level (see Materials and Methods). A: 53-year-old female, PSS; B: female, 52, PSS; C: female, 46, PSS; D: female, 43, SLE; E: female, 50, DM; F: female, 50, anaphylactoid purpura; G: female, 20, Mucha-Habermann disease; H: female, 50, livedo angititis; I: female, 58, thrombophlebitis; J: male, 41, Behçet disease; K: female, 38, pyoderma gangrenosum.

4. Clq level and clinical course

Fig. 3 presents ups and downs of Clq and C3 levels in the clinical courses of a few representative patients. In SLE accompanied by knee joint tuberculosis, the Clq level was normal in the early stage of observation, but it increased as Stevens-Johnson's syndrome appeared after streptomycin injections. On the other hand, C3 level was extraordinarily low in the early stage. After the appearance of Stevens-Johnson's syndrome, C3 rose gradually but still stayed below the normal level. The patient with mixed connective tissue disease, who had a butterfly erythema on the face, acrosclerosis, joint pain and fever, exhibited quite low levels of Clq and C3 when observation was initiated. However, as these clinical symptoms improved following steroid therapy, Clq returned to the normal level and C3 slowly rose, though not yet reaching the normal level. By contrast, in the patient with PSS, and whose clinical manifestations were stable, Clq was high and C3 and CH50 were slightly subnormal throughout the observation period. In the patient with bullous pemphigoid, Clq

was at a markedly high level at the time of hospitalization with multiple bulla, but it rapidly declined to normal as the condition improved. In the same patient, C3 remained unchanged in the normal range.

The differences in the mode of fluctuation of Clq and C3 during the observation of these representative patients seem to reflect that essentially different pathological conditions exist among the respective diseases.

5. Clq and hydroxyproline in sera

In the 11 patients with various skin diseases (shown in Fig. 4) hydroxyproline in sera and that due to Clq in the same sera were assayed. Hydroxyproline and Clq in sera were quantified by the methods referred to in Materials and Methods. Hydroxyproline due to the Clq molecule was calculated from the measured Clq amount using percentage weight of hydroxyproline. As seen from this figure, the values of these two agreed quite well with each other in the individual cases, notwithstanding the varied clinical features and/or pathological situations.

6. Fibrinogen and platelet

In 25 patients with skin diseases, Clq and fibrinogen were assayed quantitatively. The same patients were examined for platelet count at the same time (Table I). Many of those with a high serum level of Clq had a large amount of fibrinogen. On the other hand, no significant correlations were observed between Clq level and platelet count. A high platelet count was not registered in any of the cases examined.

DISCUSSION

In this study, we quantified Clq comparatively with CH50 or C3 in collagen diseases and cutaneous vasculitis. Townes (25) pointed out that the participation of antigen-antibody complexes including

Table 1. Correlation between Clq levels and fibrinogen and platelets

	Fibrinogen (180–350 mg/dl)			Platelets (15–35 × 10 ⁴)		
	High	Normal	Low	High	Normal	Low
Clq						
High	9	3	0	–	8	1
Normal	2	7	1	–	11	3
Low	2	1	0	–	2	1

complement was less prominent in PSS than in SLE, and that a low C level was found in only 2 cases out of 17 cases of the former. The results presented here show that both CH50 and C3 levels were depressed in many of the patients with either disease, but that the reduction of C3 was less prominent in PSS than in SLE. Our data are consistent with previous papers reporting low total serum C and depressed early C component levels (C1, C4 and C2) in collagen-vascular diseases (12, 21, 22). However, our findings on Clq were rather confusing. It was in fact found that Clq levels lay above the normal range in most of the patients with PSS and in a few with SLE. Moreover, the Clq level was abnormally elevated in anaphylactoid purpura and it was also raised in a majority of the patients with livedo angitis, thrombophlebitis, Mucha-Habermann disease, Behçet disease and pyoderma gangrenosum.

At least for the present the abnormal increase in Clq may possibly be explained by the following: 1) enhanced biosynthesis with the normal catabolic rate, 2) normal biosynthesis with the delayed catabolic rate or consumption, 3) leak by destruction of synthesizing and storing cells as with GOT and GPT in hepatitis or myocardial infarction, and 4) abnormal release from tissues, cells or complexes.

The first explanation might be supported by Al-Adnani & McGee's claim that Clq production would be demonstrable in pathological conditions, namely in a silica granuloma, by fibroblasts in the subcutaneous tissue of Wistar rats, but would not in normal conditions (2).

The second explanation is rather unlikely to apply, though theoretically the phenomenon cited can be caused by defect of enzyme(s) to catabolize Clq in congenital or pathological conditions, or alternatively by abnormal accumulation with the consumption of C components in the alternate pathway. The former causative factor cannot yet be ruled out on account of the lack of information, and the latter factor is still less probable, since a raised Clq level was seen quite often in vasculitis and SLE, despite C3 being within the normal range.

There is insufficient data available to discuss the third explanation in reasonable depth.

Concerning the fourth explanation, reference is made to several descriptions, as follows: The infiltration of neutrophils, associated with hypocomplementemia, is prominent in a process involving

immune complexes (22). Clq binding to immune complexes is inhibited by collagen (3, 18). Collagen-induced platelet aggregation is curbed by Clq (4, 24). The collagen-like region of Clq reacts with the platelets (26).

These previous observations do not appear to be entirely convincing, but the following speculation might be feasible. Immune complexes, deposited on blood vessel walls, activate the C system. Consequently, the fragment of C components causes the infiltration of polymorphonuclear leukocytes whose enzymes may destroy the vessel wall. The collagen released from the destroyed basal membrane or adventitia may be adsorbed onto cellular elements—including blood platelets—and occupy sites on platelets (and possibly on other cellular elements) so that Clq cannot be fixed by them. This speculation is not at variance with the report of Miller et al. (13). In this way, an increased level of Clq can be detected in blood circulation.

Serum Clq and C3 levels fluctuated independently from each other in the clinical course of various diseases (Fig. 3) and an abnormal increase in Clq was found in some diseases, which suggests that they may fulfil their own original biological and/or pathological roles in vivo besides those concerned with the enhancement of specific immunological phenomena as C components. This may be more likely in respect of Clq.

Hydroxyproline was first measured in serum and plasma by LeRoy (8) and was believed to be related to a soluble collagen component of blood. It was later found that this hydroxyproline is more likely to be associated with Clq (9). Our experiment, in which hydroxyproline and Clq were measured in the same serum, demonstrated that practically all of the serum hydroxyproline is due to Clq, even in various skin diseases. This is compatible with the observations of Miller et al. (13). It goes without saying, however, that the possibility cannot be ruled out of a tiny amount of hydroxyproline coming from collagen in serum.

We are currently continuing our efforts to ascertain a possible competition between Clq and soluble collagen or its fragments on cellular elements of human blood, using an immunohistochemical technique.

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