

DEMONSTRATION OF CIRCULATING AND TISSUE-FIXED IMMUNE COMPLEXES IN CUTANEOUS NECROTIZING VASCULITIS

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Abstract. Simultaneous demonstration of circulating and tissue-fixed immune complexes was attempted in 22 patients with cutaneous necrotizing vasculitis (7 anaphylactoid purpura, 9 cutaneous allergic vasculitis, 2 livedo reticularis, 1 thrombophlebitis, 2 erythema elevatum diutinum and 1 acute generalized pustular bacterid). In 16 out of the 22 patients, particularly patients with anaphylactoid purpura and cutaneous necrotizing vasculitis, there was a high Clq-binding activity. Decreased levels of C3 and C4 were seen in 2 and 3 patients, respectively. In 11 out of 16 skin lesions, the granular deposits of immunoglobulins and/or complement were demonstrated in the blood vessel walls of the dermis. IgA deposit was seen in anaphylactoid purpura, and IgM deposit in other types of vasculitis. C3 deposit was the most frequently noted. There was no definite correlation between Clq-binding activity and tissue deposits.

Key words: Circulating immune complexes; Tissue-fixed immune complexes; Cutaneous necrotizing vasculitis; Clq-binding activity

Although cutaneous necrotizing vasculitis varies in clinical appearance, the histological features are fairly uniform: fibrinoid degeneration of the blood vessel wall, associated with infiltration of polymorphonuclear leukocytes and nuclear dusts and with extravasation of red cells. The cutaneous manifestations—such as erythema, urticaria, papules, purpura, vesicles, pustules, nodules and ulcers—depend on the size and location in the dermis of the involved blood vessels.

Since the histological changes are similar to those induced by circulating immune complexes in animals (4), human necrotizing vasculitis is believed to be produced by immune complexes. Soluble immune complexes which are formed in the blood stream are caused to deposit in the blood vessel wall by a variety of factors. When this occurs, complement cascade may be initiated and polymorphonuclear leukocytes migrate into the blood vessel wall. Lysosomal enzymes which are released may induce a destruction of the blood vessel wall. Extravasation of red cells will follow.

We report the results of simultaneous demonstration of circulating and tissue-fixed immune complexes in various types of cutaneous necrotizing vasculitis.

MATERIALS AND METHODS

Patients

Twenty-two patients with cutaneous vasculitis (7 anaphylactoid purpura (Schönlein-Henoch), 9 cutaneous allergic vasculitis (Gougerot-Ruiter), 2 livedo reticularis, 1 thrombophlebitis, 2 erythema elevatum diutinum and 1 acute generalized pustular bacterid) were investigated. Sera and biopsy specimens were taken at the first visit or during the active stage.

Demonstration of circulating immune complexes

Clq-binding test was performed *ad modum* Zubler et al. (15). Briefly, 50 μ l of tested serum was incubated with 100 μ l of 0.2 M EDTA for 30 min at 37 °C to prevent the integration of 125 I-Clq into the intrinsic Clqrs. The mixture was then transferred to an ice bath. Fifty microlitres of diluted 125 I-Clq solution which were prepared from 250 ml of normal human blood *ad modum* Yonemasu & Stroud (14) and 1 ml of 3% polyethylene glycol were added and the mixture was left on ice for 60 min and then centrifuged. Radioactivity in the precipitate was measured and the Clq-binding activity (Clq-BA) was expressed as percent 125 I-Clq as compared with the radioactivity precipitated in a 15% trichloroacetic acid control tube.

Determination of complement

Protein concentrations of C3 and C4 in the sera were determined, using commercial plates (Hoechst). Normal ranges of C3 and C4 were 64–116 mg/dl and 17–49 mg/dl, respectively.

Demonstration of tissue-fixed immunoglobulins and complement

Biopsy specimens were cut into two. One portion was fixed in 10% formalin and stained with hematoxylin and eosin, while the other was snap-frozen in a mixture of acetone and dry ice and stored at –80 °C until use. Sections were cut in a cryostat at –20 °C and stained with FITC-

Table 1. C1q-BA, protein concentrations of C3 and C4 in the sera and direct immunofluorescence in the skin lesions

	Age	Sex	Diagnosis	C1q-BA (%)	C3 (mg/dl)	C4 (mg/dl)	Direct immuno-fluorescence
1	26	M	Anaphylactoid purpura	24.0	70.0	34.8	n.d.
2	43	M	Anaphylactoid purpura	10.8	155.5	75.0	n.d.
3	16	M	Anaphylactoid purpura	22.9	141.5	19.3	IgA(+), C3(+)
4	13	M	Anaphylactoid purpura	21.2	n.d.	n.d.	n.d.
5	46	M	Anaphylactoid purpura	33.6	n.d.	n.d.	IgA(+)
6	59	M	Anaphylactoid purpura	26.3	58.5	24.3	(-)
7	51	F	Anaphylactoid purpura	19.3	82.5	17.5	C3(+)
8	18	M	Cutaneous allergic vasculitis	15.6	142.5	75.0	M(+), C3(+)
9	33	F	Cutaneous allergic vasculitis	30.0	105.0	13.3	M(+), C3(+)
10	66	F	Cutaneous allergic vasculitis	15.6	108.0	42.3	n.d.
11	37	F	Cutaneous allergic vasculitis	19.0	105.0	39.5	M(+), C3(+)
12	58	F	Cutaneous allergic vasculitis	19.3	168.0	47.0	C3(+)
13	29	M	Cutaneous allergic vasculitis	19.9	87.0	18.4	(-)
14	17	M	Cutaneous allergic vasculitis	17.0	96.5	23.6	C3(+)
15	37	M	Cutaneous allergic vasculitis	17.9	66.5	31.4	n.d.
16	52	M	Cutaneous allergic vasculitis	27.7	64.0	26.5	M(+), C3(+)
17	43	F	Livedo reticularis	14.6	125.0	50.0	(-)
18	50	F	Livedo reticularis	11.7	85.0	29.0	(-)
19	53	M	Thrombophlebitis	18.9	97.5	13.0	n.d.
20	49	F	Erythema elevatum diutinum	14.1	61.0	36.0	M(+), C3(+)
21	63	F	Erythema elevatum diutinum	26.3	72.0	16.0	(-)
22	39	M	Acute generalized pustular bacterid	23.8	123.0	25.3	C3 (histamine injection site)
Normal range				<15.6	64.0 ~116.0	17.0 ~49.0	

conjugated anti-human IgG, IgM, IgA, C3 and C1q. Protein concentrations and F/P molar ratio of the conjugates were 4.4 mg/ml, 1.74 for anti-human IgG; 3.5 mg/ml, 1.94 for anti-human IgM; 4.1 mg/ml, 1.40 for anti-human IgA; 2.5 mg/ml, 1.54 for anti-human C3; 4.4 mg/ml, 2.12 for anti-human C1q, respectively. All conjugates were diluted to 1:10 with 0.01 M phosphate-buffered saline (pH 7.2) before use.

RESULTS

Table I shows C1q-BA, complement levels and deposits of immunoglobulins and complement in 22 patients with cutaneous necrotizing vasculitis.

The C1q-BA values of 18 healthy controls ranged from 7.0 to 15.6% (mean $\pm$ S.D. = $12.6 \pm 2.3\%$), whereas in 16 out of 22 patients the C1q-BA values were above the normal range. High C1q-BA was particularly evident in patients with anaphylactoid purpura and cutaneous allergic vasculitis (Fig. 1)

Complement levels were within normal ranges, except for a slight decrease in C3 in 1 patient each with anaphylactoid purpura and erythema elevatum diutinum and C4 in 1 patient each with cutaneous allergic vasculitis, thrombophlebitis and erythema elevatum diutinum.

The deposits of immunoglobulins and/or complement which were displayed a mostly granular pattern, were noted in the blood vessel walls of the dermis in 11 out of 16 tested specimens. IgA deposit was demonstrated in 2 patients with anaphylactoid purpura, whereas IgM deposit was observed in 5 patients with other types of vasculitis. C3 was detected in 10 patients of both groups. Neither IgG nor C1q was demonstrated in any case.

DISCUSSION

A variety of methods has been employed for the demonstration of the circulating immune complexes. In the present study, we used the C1q-binding test and found an elevation of C1q-BA in 16 out of 22 patients with cutaneous necrotizing vasculitis. High C1q-BA was particularly noted in patients with anaphylactoid purpura and cutaneous allergic vasculitis. Although this method detects not only circulating immune complexes, but also other substances such as aggregated γ -globulin, DNA and lipopolysaccharide, it is likely that immune complexes exist in the majority of patients with cutane-

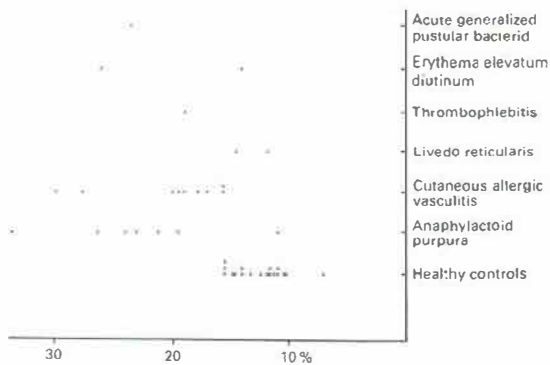


Fig. 1. C1q-binding activity (%).

ous necrotizing vasculitis, since 18 healthy control subjects did not have such high C1q-BA values.

Asghar et al. (1) used the agarose gel diffusion plate and found C1q-reactive material in the sera of 2 patients with allergic vasculitis. Braun-Falco et al. (2) described the presence of circulating immune complexes in all 6 patients with allergic vasculitis and 2 of 3 patients with livedo vasculitis, using the ^{125}I C1q deviation test. Using the platelet aggregation test, we reported the presence of immune complexes in the sera of 4 of 8 patients with cutaneous allergic vasculitis (13). These reports are consistent with our present results. Recently, Tappeiner & Wolff described the elevation of C1q-BA levels in patients with systemic, necrotizing vasculitis, in contrast to normal C1q-BA in patients with non-systemic necrotizing vasculitis and lymphocytic vasculitis (11).

The reason why the number of sera showing a depressed level of complement was so small is not clear. A relatively small amount of immune complexes in the sera of patients with necrotizing vasculitis, as compared with those of patients with systemic lupus erythematosus, or the small capability of immune complexes to activate complement may be responsible.

The granular deposits of immunoglobulins, associated with C3, in the blood vessel walls are highly suggestive evidence of tissue-fixed immune complexes. Although some authors (6, 9, 10) reported a predominance of IgG as the deposited immunoglobulin, a recent report by Sams et al. (8) stated that IgM and IgA were two major immunoglobulins. Our present study has confirmed this. It was of interest that IgA deposits were noted only in patients with anaphylactoid purpura, and IgM in pa-

tients with other types of vasculitis. C3 was the most frequently noted, and this may be due to the amplification effect (8).

The reason why IgA was frequently demonstrated in patients with anaphylactoid purpura has not been elucidated. Trygstad & Stiem (12) reported a high level of IgA in sera from 10 of 20 patients with anaphylactoid purpura in whom onset came within 3 months. We found a high level of serum IgA (1784 mg/dl) in 1 of 3 tested sera of patients with anaphylactoid purpura.

There was no definite correlation between high C1q-BA and tissue deposits. This may be due to the time of biopsy and the sampling sera. Sams et al. (8) reported the higher frequency of demonstrated deposited immunoglobulins in uninvolved skin than in involved skin, and Braverman & Yen (3) and Gower et al. (5) demonstrated immunoglobulins and complement in the injection sites of histamine within 3–4 hours. We too demonstrated C3 deposit in a patient with acute generalized pustular bacterid only after an injection of histamine.

Recently, we reported the presence of circulating immune complexes and their deposition in the blood vessel walls in the upper dermis of some patients with erythema multiforme, although the positive case was less often found than in the present study (7). We speculated on the transient production of circulating immune complexes and their deposition in the development of erythema multiforme. On the other hand, the intermittent formation of circulating immune complexes and their deposition in the tissue may be responsible for the intermittent appearance of the eruption in cutaneous necrotizing vasculitis. However, further investigations are required before our speculation can be confirmed.

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REFERENCES

1. Asghar, S. S., Faber, W. R. & Cormane, R. H.: C1q precipitin in the sera of patients with allergic vasculitis (Gougerot-Ruiter syndrome). *J Invest Dermatol* 64: 113, 1975.
2. Braun-Falco, O., Männel, C. & Scherer, R.: Nach-

- weis von zirkulierenden löslichen Immunkomplexen im Serum von Psoriasispatienten mit dem ^{125}I -C1q-Ablenkungstest. *Hautarzt* 28: 658, 1977.
3. Braverman, I. M. & Yen, A.: Demonstration of immune complexes in spontaneous and histamine-induced lesions and in normal skin of patients with leukocytoclastic angiitis. *J Invest Dermatol* 64: 105, 1975.
 4. Cochrane, C. G.: Mechanisms involved in the deposition of immune complexes in tissues. *J Exp Med* 134: 75 s, 1971.
 5. Gower, R. G., Sams, W. M., Thorne, E. G., Kohler, P. F. & Clayman, H. N.: Leukocytoclastic vasculitis: sequential appearance of immunoreactants and cellular changes in serial biopsies. *J Invest Dermatol* 69: 477, 1977.
 6. Handel, D. W., Roenigk, H. H., Shainoff, J. & Deodhar, S.: Necrotizing vasculitis. Etiologic aspects of immunology and coagulopathy. *Arch Dermatol* 111: 847, 1975.
 7. Imamura, S., Yanase, K., Taniguchi, S., Ofuji, S. & Mangaoil, L.: Erythema multiforme: demonstration of immune complexes in the sera and skin lesions. *Br J Dermatol* 102: 161, 1980.
 8. Sams, W. M., Claman, H. N., Kohler, P. F., McIntosh, R. M., Small, P. & Mass, M. F.: Human necrotizing vasculitis: Immunoglobulins and complement in vessel walls of cutaneous lesions and normal skin. *J Invest Dermatol* 64: 441, 1975.
 9. Schroeter, A. L., Copeman, P. W. M., Jordon, R. E., Sams, W. M. & Winkelmann, R. K.: Immunofluorescence of cutaneous vasculitis associated with systemic disease. *Arch Dermatol* 107: 254, 1971.
 10. Stringa, S. G., Bianchi, C., Casala, A. M. & Bianchi, O.: Allergic vasculitis Gougerot-Ruiter syndrome. *Arch Dermatol* 95: 23, 1967.
 11. Tappeiner, G. & Wolff, K.: Zirkulierende Immunkomplexe und nekrotisierende Vaskulitis. *Hautarzt* 30: 628, 1979.
 12. Trygstad, C. W. & Stiehm, E. R.: Elevated serum IgA globulin in anaphylactoid purpura. *Pediatrics* 47: 1023, 1971.
 13. Yanase, K. & Imamura, S.: Detection of circulating immune complexes in some skin diseases by platelet aggregation test. *Br J Dermatol* 100: 227, 1979.
 14. Yonemasu, K. & Stroud, R. M.: C1q: rapid purification method for preparation of monospecific antisera and for biochemical studies. *J Immunol* 106: 304, 1971.
 15. Zubler, R. H., Lange, G., Lambert, P. H. & Miesher, P. A.: Detection of immune complexes in unheated sera by a modified ^{125}I -C1q binding test. *J Immunol* 116: 232, 1975.

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