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# Membrane attack complex of complement in Henoch-Schönlein purpura skin and nephritis

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**Summary. The present study using direct immunofluorescence with monoclonal antibodies to C5b-9 complex-related antigens was undertaken to determine whether complement activation in Henoch-Schönlein purpura (HSP) causes assembly of the membrane attack complex of complement (MAC) in skin and nephritis lesions. The deposition of C5, C6, C7,C8, C9, and C5b-9 neoantigens was noted in the vascular walls of papillary dermis and/or subpapillary dermal plexus of the vessels in 11 out of 15 patients with HSP. Their presence in vessel walls indicates complement activation which leads to terminal complement activation. There were small deposits of S protein at the same sites in three of the 11 skin specimens. Thus, the majority of C5b-9 demonstrated in HSP skin was the cytolytically active C5b-9 complex, MAC. Granular deposits of C5b-9 related antigens without S protein were also found in the capillary walls and mesangium of the glomeruli of two out of four specimens from patients with HSP nephritis; in the other two S protein was colocalized with the deposition of C5b-9. The results of the present study indicate that complement activation leading to generation of MAC may possibly be involved in the pathogenesis of vascular injury in a significantly large number of skin lesions and of HSP nephritis.**

**Key words:** Henoch-Schönlein purpura – Complement – Cytolysis – Endothelial cell damage

Henoch-Schönlein purpura (HSP) is a generalized vasculitis of unknown etiology. Histopathologically, small cutaneous vessels show leukocytoclastic vasculitis (LCV), a pathological condition characterized by infiltration of polymorphonuclear leukocytes (PMNLs) in and around small blood vessels, nuclear dust, hemorrhage, and necrosis of blood vessels. It has been hypothesized that the pathogenesis involves the formation of circulating antigen-antibody complexes, which are then deposited in small blood vessels, followed by inflammation. This theory is based on the following: (1) IgA-containing circulating immune complexes (IgA-CIC) are present in the active phase of HSP [13, 14]; we previously showed good correlation between IgA-CIC levels and clinical events of cutaneous and systemic organ involvements of HSP [11]. (2) The pathological features of LCV closely resemble those in the Arthus phenomenon, in which interactions of antigen and antibody, followed by complement activation, lead to inflammation changes in vessels [19]. (3) By immunofluorescence (IF) microscopy, deposits of immunoglobulins, predominantly of the IgA class, and C3 complement component have often been seen in cutaneous vessel walls and glomeruli [1]. It is generally accepted that these immune complexes, fixed to vascular walls, locally activate the complement system, with elaboration of chemotactic factors for PMNLs. PMNLs may subsequently cause damage to vascular structures through the action of their lysosomal enzymes. There is also the theoretical possibility that the complement may have an alternate role, such as a direct cytotoxic effect on the vascular endothelium. The present study using carefully characterized monoclonal antibodies to C5,C6, C7, C8, C9, and C5b-9 neoantigens and S protein, was conducted to determine whether the membrane attack complex of complement (MAC) is present in skin and glomerular lesions of HSP which may develop into typical LCV.

## Materials and methods

### *Patients and biopsy materials*

All patients had active lesions on the skin when biopsy specimens were obtained. Fifteen patients with HSP were classified according to clinical and histopathological findings. Four patients, nos. 1–4, developed nephritis as confirmed by microscopical urinary abnormality, proteinuria, and histopathological evidence of nephritis. Biopsy specimens were obtained from skin lesions of all 15 patients and from the kidneys of four patients with HSP nephritis. The controls for skin lesions consisted of 5 healthy volunteers and 5

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**Table 1.** Immunofluorescence and histological findings on Henoch-Schönlein purpura (HSP) skin

| Case | IF[a] | | | | | | PMNL infiltration[b] | | Vascular damage[c] | |
|---|---|---|---|---|---|---|---|---|---|---|
| | IgA | IgG | IgM | C3c | C5b-9[d] | S protein | P | S | P | S |
| HSP | | | | | | | | | | |
| 1 | 2+ | − | 1+ | 1+ | 2+/ps | 1+ | − | 2+ | 1+ | 2+ |
| 2 | 1+ | − | 1+ | 1+ | 1+/p | − | 1+ | 3+ | 1+ | 1+ |
| 3 | 2+ | − | 2+ | 2+ | 1+/p | − | − | 1+ | 1+ | 1+ |
| 4 | 2+ | − | 1+ | 3+ | 3+/p | 1+ | − | 2+ | 2+ | 3+ |
| 5 | 2+ | − | 2+ | 3+ | 2+/ps | 1+ | − | 1+ | 1+ | 1+ |
| 6 | 1+ | − | − | 2+ | 2+/p | − | 1+ | 3+ | 2+ | 2+ |
| 7 | − | − | − | − | 2+/ps | − | − | 1+ | 2+ | 2+ |
| 8 | 1+ | − | − | 3+ | 2+/p | − | − | 2+ | 1+ | 1+ |
| 9 | 2+ | 1+ | − | 2+ | 2+/p | − | − | 1+ | 1+ | 1+ |
| 10 | − | − | − | 2+ | 2+/ps | − | − | 2+ | 2+ | 2+ |
| 11 | − | − | − | 2+ | 2+/ps | − | − | 2+ | 2+ | 2+ |
| 12 | − | − | − | − | − | − | − | 1+ | − | 1+ |
| 13 | − | − | − | − | − | − | − | 1+ | − | 1+ |
| 14 | − | − | − | − | − | − | − | 1+ | − | 1+ |
| 15 | − | − | − | − | − | − | − | 1+ | − | 1+ |
| Controls | | | | | | | | | | |
| 1−21 | − | − | − | − | − | | | | | |

[a] Intensity of IF staining: −, no fluorescence; 1+, weak granular deposits; 2+, marked granular deposits; 3+, extensive granular deposits. Deposition sites: p, extensively papillary dermal vessels (capillary loops); ps, subpapillary plexus of vessels with involved papillary dermal vessels

[b] Degree of cell infiltration and

[c] endothelial cell injury: −, minimal; 1+, mild; 2+, marked; 3+, severe. Sites of cell infiltration/dermal vessels damaged: P, papillary dermal vessels (capillary loops); S, subpapillary plexus of the vessels

[d] C5b-9 complex was identified by the presence together of C5, C6, C7, C8, C9, and C5b-9 neoantigens

patients with erythema multiforme, 5 with erythema nodosum, 5 with urticaria, and 1 with erythema due to sepsis. The control patients were also in the active phase. Control kidney specimens were taken from three patients with lipoid nephrosis. Part of each specimen obtained from the patients and controls was fixed in formalin, and sections of paraffin-embedded tissue were stained with hematoxylin and eosin for routine histopathological examination. The remaining tissue was snap-frozen in liquid nitrogen and stored at −70° C until use.

### *Monoclonal antibodies to late complement components and MAC related neoantigens*

Murine monoclonal antibodies (tissue culture supernatants) to human complement components C5, C6, C7, C8, C9 and C5b-9 neoantigens were kindly provided by Cytotech (San Diego, Calif.). These antibodies were derived from splenocytes removed from a BALB/c mouse immunized with highly purified human SC5b-9 and have been characterized in detail elsewhere [20]. Briefly, the monoclonals to C5, C6, C7, C8, and C9 reacted with individual purified components in their unassembled form and showed positive reactions with the assembled human C5b-9 complex on rabbit red cell ghost. The anti-C5b-9 neoantigen does not bind to any complex precursor or S protein, but does bind to human SC5b-9, which was used by Cytotech to immunize the mice, and to neoantigens of the assembled human C5b-9 complex on rabbit red cell membranes. The mouse monoclonal antihuman S protein antibody was also obtained from Cytotech.

### *Immunofluorescence procedures*

Cryosat sections (4 μm) of the specimens were mounted on glass slides followed by incubation with monoclonal antibodies to C5, C6, C7, C8, C9, and C5b-9 neoantigens or S protein for 30 min and then washed. In addition, the murine monoclonal antihuman C3c (Cytotech) was used in place of monoclonal antibodies to terminal complement components. Fluorescein isothiocyanate (FITC) conjugated goat antimouse IgG (Cappel Laboratories, Cochranville, Pa.), previously absorbed with normal human gamma globulin coupled to cyanogen bromide activated Sepharose 4B-CL, was then added followed by incubation for an additional 30 min. Other sections were similarly treated with FITC-labeled goat antihuman IgG, IgM, and IgA (Cappel Laboratories).

### *Raji cell radioimmunoassay (RIA)*

The levels of IgA-CIC and IgG-CIC in the sera from patients with HSP nephritis were determined according to the modified Raji cell RIA as previously described by us [11]. The amounts of IgA-CIC and IgG-CIC in each serum tested were expressed as equivalent concentrations of aggregated IgA and IgG (μg/ml of serum), respectively.

### *Statistical analysis*

Student's *t*-test was used to evaluate the differences among the mean values of each group of serum samples.

## Results

### *Immunoglobulin and complement deposits in HSP*

The immunohistological findings are summarized in Table 1. IgA was predominantly of the immunoglobulin

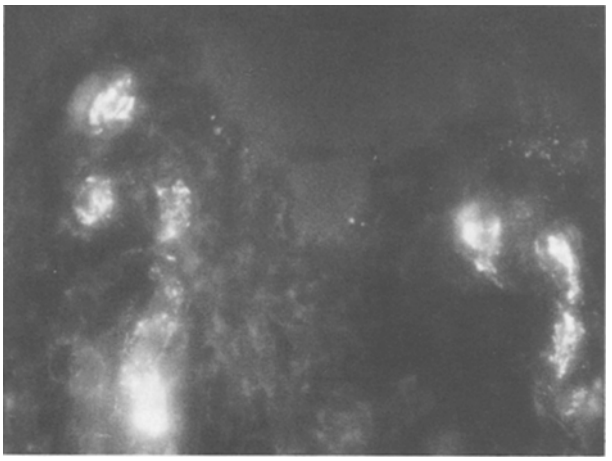

**Fig. 1.** Extensive granular deposits of C5b-9 neoantigens on vascular walls of dermal vessels of Henoch-Schönlein purpura skin. Original magnification, ×320

class which was deposited on the vessel walls of the skin lesions. C3c was present in the vascular walls of these lesions in 10 out of 15 cases. Staining with anti-C5, -C6, -C7, -C8, -C9, and -C5b-9 neoantigens indicated granular deposits in 11 of the 15 specimens (73%; Fig. 1). In some cases deposits could be observed only on papillary dermal vessels (p), while in others deposits were found both there and on a subpapillary plexus of vessels (ps) (p, 6 of the 11 C5b-9 complex-positive specimens, ps, 5 of the specimens). In eight cases the C5b-9 complex was present at the corresponding sites of IgA and C3c deposition. In two cases (nos. 10 and 11), C3c and the C5b-9 complex were present together without IgA deposition and in one case (no. 7), only the C5b-9 complex could be seen. Slight deposits of S protein were observed at the same sites as the C5b-9 deposits in three cases. (nos. 1, 4, and5), but not at all in the other eight C5b-9 positive cases. Although C3c deposition was noted predominantly in papillary dermal vessel walls, PMNLs were shown histopathologically to have infiltrated the subpapillary plexus of the vessels although being sparse or even absent in the papillary dermis except for in patients 2 and 6. As noted for the subpapillary plexus of the vessels, the vascular endothelium of papillary dermal vessels in each C5b-9 complex-positive case appeared edematous, necrotic, and, on some occasions, the cells became disengaged from the lumen and were no longer present. Red blood cells (RBCs) extravasated. The vascular structures were essentially intact when there was no deposition of immunoglobulin, C3c, or C5b-9 complex (patients 12–15). In the control group, no deposits were found in the skin of healthy volunteers or patients with various skin disorders.

Table 2 shows the immunohistological findings for biopsied kidney specimens from four patients with HSP nephritis. IgA deposition was noted in all four cases and IgM deposits as well. The deposition patterns were granular in the glomerular capillary walls and mesangium. The glomeruli in all four cases were granularly stained with anti-C3c along the peripheral capillary walls. The deposition of C5b-9-related antigens, with (two cases) or without S protein (two cases) was also present (Fig. 2). All specimens from lipoid nephrosis as the control were negative with immunoglobulin, complement, and S-protein staining.

**Table 2.** Immunofluorescence findings[a] on Henoch-Schönlein purpura (HSP) nephritis

| Case | IgA | IgG | IgM | C3c | C5b-9 | S protein |
|---|---|---|---|---|---|---|
| HSP nephritis | | | | | | |
| 1 | 2+ | – | 2+ | 1+ | 2+ | + |
| 2 | 2+ | – | 2+ | 1+ | 2+ | – |
| 3 | 1+ | – | 2+ | 1+ | 2+ | – |
| 4 | 2+ | – | 1+ | 1+ | 3+ | + |
| Lipoid nephrosis | | | | | | |
| 1 | – | – | – | – | – | – |
| 2 | – | – | – | – | – | – |
| 3 | – | – | – | – | – | – |

[a] Intensity of IF staining: –, no fluorescence; 1+, weak granular deposits; 2+, marked granular deposits; 3+, extensive granular deposits

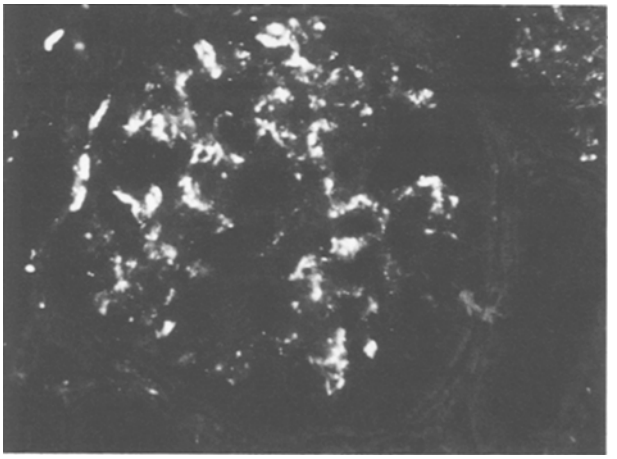

**Fig. 2.** Extensive granular deposits of C5b-9 neoantigens along peripheral capillary walls of glomeruli of Henoch-Schönlein purpura nephritis. Original magnification, ×320

**Table 3.** Circulating immune complexes (CICs) in patient sera of Henoch-Schönlein purpura (HSP) nephritis

| | IgA-CIC | | IgG-CIC |
|---|---|---|---|
| HSP nephritis | 286.5 ± 173.8 ($n = 4$) | $p < 0.05$ | 6.7 ± 4.7 ($n = 4$) |
| Normal donors | 7.1 ± 9.2 ($n = 12$) | | 4.3 ± 5.2 ($n = 18$) |

Values are mean ± SD (μg/ml)

*Circulating immune complexes in patient sera* (Table 3)

The mean level of IgA-CIC in 12 specimens of normal human serum (NHS) was 7.1 ± 9.2 μg/ml, the upper limit

(mean + 2 SD) for the test being 25.5 μg/ml. An examination of 18 NHS samples showed the mean level of the normal range of IgG-CIC to be 4.3 ± 5.2 μg/ml and the upper normal limit (mean + 2 SD), 14.7 μg/ml. Thus, in all four patients with HSP nephritis, IgA-CIC was positive with a mean value (286.5 ± 173.8 μg/ml) significantly higher than that of the normal control level ($p < 0.05$). In contrast, the IgG-CIC level (6.7 ± 4.7 μg/ml) was not statistically different from the normal level.

## Discussion

When terminal complement components assemble to form the C5b-9 complex, new antigens not normally present on individual components are produced [7]. The monoclonal antibodiy used in this study (anti-C5b-9 neoantigens) specifically detects one of the new antigenic determinants on the assembled C5b-9 complex. The deposition of late complement components (C5, C6, C7, C8, C9) and C5b-9 neoantigens on small vessel walls of either the subpapillary dermal plexus of the vessel or the papillary dermis of skin lesions was found in 11 of 15 patients with HSP. The deposition was independent of the colocalization of S protein, exept for three specimens in which there was slight depositon of S protein. Terminal complement activation without S protein is an indication of the presence of the cytotoxic complement C5b-9 complex, MAC, at these sites. Our findings thus demonstrate the complete activation of the complement system leading to assembly of MAC in most skin lesions of HSP, and provide additional confirmation of the results of Boom et al. [4] who consider MAC to be a causative factor in LCV. The vascular endothelium of dermal vessels in each MAC-positive case was injured. It is noteworthy that this damage was evident even in papillary dermal vessels in which no PMNL infiltration could be noted but C5b-9 deposition was seen. Thus, MAC may be necessary for the occurrence of endothelial cell damage. The assembly of MAC on RBC membranes following complement activation results in lysis [15]. Nucleated cells were generally more resistant to complement-mediated cytolysis by the rapid removal of C5b-9 channels from the cell surface than are RBCs. However, under certain conditions, complement will damage the membranes of nucleated cells. For instance, either a large amounts of MAC deposition on the cell surface or blockade of the lipid metabolism of the cells results in increased susceptibility to complement killing (for review, see [17]). MAC has recently been proposed as a pathogenic factor in certain cutaneous diseases such as systemic lupus erythematosus [3], bullous pemphigoid [5], dermatitis, herpetiformis [6], LCV [4], and pemphigus vulgaris and pemphigus foliaceus [12]. Our previous studies showed that activation of human complement by pemphigus antibody results in cytotoxicity by significantly altering the permeability of murine epidermal cell membranes to ethidium bromide [10] and that this is sufficient for bringing about the detachment of cells in vitro [9]. MAC may also serve indirectly as a modulator of immunological and inflammatory reactions. In vitro experiments have recently indicated that terminal complement components enhance the antigenic and functional expression of C5a [8] and stimulate the release of arachidonates from various cells [17]. These may possibly lead to further inflammation.

The glomerular deposition of C5, C6, C7, C8, C9, and C5b-9 neoantigens was also found in specimens from four patients with HSP nephritis, implicating complement membrane attack in the pathogenesis of this disease. This confirms the previous work of Rauterberg et al. [16] which demonstrated the deposition of C5b-9 in IgA nephropathy and HSP nephritis.

In three skin tissues and two glomerular tissues, S protein was shown to be colocalized with C5b-9. S protein is a plasma protein capable of binding to the C5b-7 complex during its assembly, resulting in the formation of SC5b-9 [2]. The SC5b-9 complex is a cytolytically inactive complex since it is not capable of penetrating a cell membrane if assembled in the fluid phase. So far, however, the significance of the presence of SC5b-9 in situ is not fully understood. In a Raji cell assay increased levels of IgA-CIC were found in the sera of four patients with HSP nephritis. This assay can detect the complement fixing-immune complex in the circulation [18]. Terminal complement sequences may possibly be activated by immune complexes in the circulation and S protein may bind to them, resulting in the formation of the SC5b-9 complex. This complex may then passively be trapped in cutaneous and glomerular vessels in the circulation. Such SC5b-9 (SC5b-9 in fluid phase) would be cytolytically inactive. However, there is the possibility that both MAC and the inactive SC5b-9 complex are generated in situ or that S protein binds to the active C5b-9 complex already assembled on the cell membrane (SC5b-9 complex in situ). In such an event, complement membrane attack may also possibly be a cause of endothelial damage in HSP and this may be sufficient to explain why, in the three skin specimens with positive SC5b-9, small cutaneous vessels were injured, as also noted for the C5b-9-positive specimens without S protein. Additional studies need to be undertaken to clarify these questions.

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## References

1. Baart de la Faille-Kuyper EM, Kater L, Kooiker CJ, Mees EJD (1973) IgA-deposits in cutaneous blood vessel walls and mesangium in Henoch-Schönlein syndrome. Lancet I:892–893
2. Bhakdi S, Traum-Jensen J (1982) Terminal membrane C5b-9 complex of human complement: transition from an amphiphilic to a hydrophilic state through binding of the S protein from serum. J Cell Biol 94:755–759
3. Biesecker G, Lavin L, Ziskind M, Koffler D (1982) Cutaneous localization of the membrane attack complex in discoid and systemic lupus erythematosus. N Engl Med J 306:264–270
4. Boom BW, Out-Luiting CJ, Baldwin WM, Westedt M-L, Daha MR, Vermeer B-J (1987) Membrane attack complex of com-

plement in leukocytoclastic vasculitis of the skin. Arch Dermatol 69:477–484
5. Dahl MV, Falk RJ, Carpenter R, Michael AF (1984) Deposition of the membrane attack complex of complement in bullous pemphigoid. J Invest Dermatol 82:132–135
6. Dahl MV, Falk RJ, Carpenter R, Michael AF (1985) Membrane attack complex of complement in dermatitis herpetiformis. Arch Dermatol 121:70–72
7. Falk RJ, Dalmasso AP, Kim Y Tsaich, Scheinman JI, Gewurz H, Michael AF (1983) Neoantigen of the polymerized ninth component of complement. Characterization of a monoclonal antibody and immunohistochemical localization in renal disease. J Clin Invest 72:560–573
8. Gresham HD, Renfer L, Hammer CH, Frank MM (1987) Terminal complement components play a role in the expression of C5a. J Immunol 138:838–841
9. Kawana S, Geoghegan WD, Jordon RE (1985) Complement fixation by pemphigus antibody. II. Complement enhanced detachment of epidermal cells. Clin Exp Immunol 61:517–525
10. Kawana S, Geoghegan WD, Jordon RE (1986) Complement fixation by pemphigus antibody. III. Altered epidermal cell membrane integrity mediated by pemphigus antibody and complement. J Invest Dermatol 86:29–33
11. Kawana S, Ohta M, Nishiyama S (1986) Value of the assay for IgA-containing circulating immune complexes in Henoch-Schönlein purpura. Dermatologica 172:245–253
12. Kawana S, Geoghegan WD, Jordon RE, Nishiyama S (1989) Deposition of the membrane attack complex of complement in pemphigus vulgaris and pemphigus foliaceus skin. J Invest Dermatol 92:588–592
13. Kauffmann RH, Herrmann WA, Meyer CJLM, Daha MR, Van Es LA (1980) Circulating IgA-immune complexes in Henoch-Schönlein purpura. A longitudinal study of their relationship to disease activity and vascular deposition of IgA. Am J Med 69:859–866
14. Levinsky RJ, Barratt TM (1979) IgA immune complexes in Henoch-Schönlein purpura. Lancet II:1100–1103
15. Müller-Eberhard HJ (1985) The killer molecule of complement. J Invest Dermatol 85:47s–52s
16. Rauterberg EW, Lieberknecht HM, Wingen AM, Ritz E (1987) Complement membrane attack (MAC) in idiopathic IgA-glomerulonephritis. Kidney Int 31:820–829
17. Shin ML, Carney DF (1988) Cytotoxic action and other metabolic consequences of terminal complement proteins. Prog Allergy 40:44–81
18. Theofilopoulos AN, Wilson CB, Dixon FJ (1976) The Raji cell radioimmunoassay for detecting immune complexes in human sera. J Clin Invest 57:169–182
19. Udaka K (1971) The Arthus reaction. In: Movat HZ (ed) Inflammation, immunity and hypersensitivity. Harper and Row, New York, pp 399–416
20. Xia P, Jordon RE, Geoghegan WD (1988) Complement fixation by pemphigus antibody. V. Assembly of the membrane attack complex on cultured human keratinocytes. J Clin Invest 82:1939–1947