Formation of Soluble Immune Complexes by Complement in Sera of Patients with Various Hypocomplementemic States

Difference between Inhibition of Immune Precipitation and Solubilization

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Abstract

To examine whether the ability of complement to form soluble immune complexes plays a role in preventing immune complex-mediated diseases, we analyzed the capacity of complement to inhibit immune precipitation (IIP) and to solubilize preformed immune aggregates (SOL) in 23 sera of patients with various hypocomplementemic states, and we correlated the results of these studies with the clinical syndromes found in the various patients.

In sera with deficiency or depletion of early classical pathway components, IIP was profoundly altered, whereas SOL was delayed but in the normal range. In contrast, in sera with C3 depletion but intact classical pathway and in properdin-deficient serum, IIP was initially preserved, whereas SOL was abolished.

Since the incidence of immune complex diseases in various hypocomplementemic states correlates with the severity of IIP defects, but not with reduced SOL, it is suggested that IIP is an essential biological function of complement that prevents the rapid formation of insoluble immune complexes in vivo.

Introduction

The association between complement deficiency and immune complex diseases has become evident over recent years (1). It is therefore possible that complement has a physiological role in preventing tissue formation or deposition of immune complexes. In vitro, complement can bring about the formation of soluble complexes either by inhibiting their aggregation at the time of the antigen-antibody reaction or by solubilizing preformed immune precipitates (2, 3). Inhibition of immune precipitation (IIP)[1] relies predominantly on an intact classical pathway of complement activation, whereas solubilization (SOL) is dependent on the presence of normal alternative pathway function (4–6). Both processes result in the formation of soluble complexes by the covalent binding of C3 fragments (7, 8).

Assays have been devised to measure SOL and IIP in vitro in sera of patients with various immune complex-mediated diseases (9–22). Abnormalities have been found in different diseases, but no attempts have been made to compare these two functions in defined situations in order to determine their biological relevance.

In this study, IIP and SOL were analyzed and compared in various hypocomplementemic sera. The results indicated that alterations in IIP and SOL differ depending on the type of hypocomplementemia. In particular, assays for IIP revealed rapid and immediate aggregation of complexes in sera with early classical pathway defects, suggesting that such hypocomplementemic states are a favorable ground for the formation of insoluble complexes.

Methods

Immune complex model

A ^{125}I-bovine serum albumin (BSA; Armour Ltd., Eastbourne, United Kingdom)-rabbit anti-BSA antibody model was used for both SOL and IIP as previously described (3, 6). Briefly, the IgG fraction of rabbit BSA antiserum was prepared by Na_2SO_4 salt precipitation. Antibodies cross-reacting with human serum albumin (HSA) were removed by immunoadsorption on insolubilized HSA (Sigma Chemical Co., St. Louis, MO). Thereafter, the rabbit anti-BSA antibody did not react with chloramine T ^{125}I-radiolabeled HSA (23), i.e., did not form soluble or precipitating immune complexes as determined by sucrose density gradient ultracentrifugation (SDGU).

(a) IIP assay. 50 ml of test serum, 0.5 μg ^{125}I-BSA, and when required, the appropriate additional reagent, were mixed and adjusted to a final volume of 120 μl with buffer (complement-fixing diluent (CFD): barbital-buffered saline, pH 7.4, conductivity 14 mmHo; CFD, Oxoid Ltd., Basingstoke, United Kingdom). After a preincubation of 5 min at 37°C, 9 μg of rabbit anti-BSA antibody was added and the incubation carried out at 37°C. 25-ml aliquots were removed after various time intervals, mixed with 1 ml phosphate-buffered saline (PBS) (pH 7.4, conductivity 14 mmHo; PBS, Oxoid Ltd.) and centrifuged at 3,000 *g* for 10 min at 4°C. Supernatants and pellets were separated and the radioactivity measured.

(b) SOL assay. Immune precipitation of ^{125}I-BSA-rabbit anti-BSA antibody was carried out for 1 h at 37°C and 4 h at 4°C in CFD. By that time, 95% of the complexes had precipitated when centrifuged at 3,000 *g* for 10 min. These immune aggregates were used directly without centrifugation in the assay at 1/5 of the concentration used in IIP, i.e., 0.1 μg ^{125}I-BSA plus 1.8 anti-BSA antibody. The mixtures containing 50 μl of test serum and the immune aggregates were adjusted to a final volume of 120 μl with CFD. The assay was carried out similarly to IIP.

The immune complex load was halved in IIP and SOL experiments using properdin (P) and C4-deficient sera. The various sera were stored at −70°C before performing the assays except for the five sera containing

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1. *Abbreviations used in this paper:* AP, alternative pathway; C1-In, C1 inactivator; CFD, complement-fixing diluent; def(s), deficiency; HSA, human serum albumin; IIP, inhibition of immune precipitation; NHS, normal human serum; P, properdin; SDGU, sucrose density gradient ultracentrifugation; SOL, solubilization.

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cryoglobulins. These sera were studied fresh: blood was allowed to clot for 15 min at 37°C; the serum separated by centrifugation at 37°C was immediately used in the assays. 10 mM etylene diamine tetraacetate (EDTA) was used to block all complement function, and 10 mM etylene glycol tetraacetate (EGTA) plus 2 mM Mg was used to block classical pathway function. All assays were done in duplicate.

SDGU

50 ml of the mixtures (IIP and SOL assays) were incubated for 2 h and overlayered onto a 10–50% (wt/wt) sucrose gradient in PBS and ultracentrifuged at 80,000 *g* for 12.5 h at 4°C. The polystyrene centrifuge tubes and the 24 fractions collected were counted and the percentage of the total radioactivity determined for each fraction. Radiolabeled IgM served as a 19-S (Svedberg unit) marker.

Purified complement proteins

C1q and C4 were purified according to published methods (24, 25). Properdin was purified as described by Medicus et al. (26). The purity of these three proteins was shown by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in the presence and in the absence of reducing agents (27). The P concentration was measured by single radial immunodiffusion using a monospecific antiserum (28), and the result expressed in percentage of a normal human serum pool obtained from 25 donors. The C1q and C4 concentrations and functional activities were determined, respectively, by the method of Lowry (29) using BSA as a standard, and by hemolytic functional assays (30, 31). The specific activity was 100% for C1q and 75% for C4 when compared with C1q and C4 of the plasma used for their purification.

Complement, immune complex, and cryoglobulin measurements

All sera were stored at −70°C until tested, except the five sera containing cryoglobulins, which were tested fresh. C1 inactivator (C1-In), C4, C3, and C2 were measured by single radial immunodiffusion using monospecific antisera (Miles Laboratories, Inc., Elkhart, IN) according to the manufacturer's notice. C1q was measured according to Ziccardi and Cooper (32) using anti-C1q antiserum raised in a goat by three injections at 2-wk interval of purified C1q in Freund's adjuvant (Difco Laboratories, Detroit, MI) (complete adjuvant for the first injection, incomplete adjuvant for the two following injections). C4-binding-protein measurements have been described (33). CH_{50}, factor B, and alternative pathway (AP) activity were determined by hemolytic plate assays (34). Functional C4 was measured (31) and compared with antigenic concentrations. All results were expressed in percentage of a normal human serum (NHS) pool collected from 25 blood donors. C1q binding assays for immune complexes were carried out as described by Zubler et al. (35). Cryoglobulins were detected by incubation of serum for 96 h at 4°C and their content then analyzed (36).

Results

IIP and SOL assays were standardized. Using the ^{125}I-BSA–rabbit anti-BSA model described, normal serum inhibited immune precipitation completely over the period of time studied (2 h)—i.e., >94% soluble complexes in all 25 normal sera tested (Fig. 1 *A*). The immune complex concentrations used in the assay corresponded to half the maximum concentration that could be maintained in solution in NHS; thus, any decrease in IIP was abnormal. When 10 mM EDTA was added to block complement function, IIP was abolished and complexes aggregated rapidly so that precipitation was nearly complete in 30 min.

Normal serum solubilized 55% (±25%) preformed immune aggregates in 2 h (normal means±2 SD of 25 normal sera) (Fig. 1 *B*). SOL was abolished in the presence of 10 mM EDTA.

The formation of soluble immune complexes by IIP and SOL was studied in various hypocomplementemic sera (Table I). These sera were grouped according to their main complement defect: (*a*) deficiencies (def) of early classical pathway components (15 sera); (*b*) depletion of C3 and properdin def (5 sera); and (*c*) def of one of the membrane attack complex proteins (3 sera). Immune complexes were measured in all sera, since their presence could interfere with normal complement function. C1q binding material was found in the five patients with cryoglobulinemia (>50% binding), and in C1q and C4 deficient sera (84 and 12% binding, respectively) (normal: <5%).

IIP and SOL assays in sera with depletion or def of early classical pathway components. The major observation was a severe defect in IIP. Initial fast precipitation was evident in most sera, and this was followed by partial redissolution of the complexes (Fig. 1). A similar kinetic was observed in normal serum chelated with 10 mM EGTA plus 2 mM Mg, indicating that immediate aggregation was related to the absence of classical pathway function. However, IIP remained normal in two sera: these two exceptions were the sera with C1 inactivator deficiency but without severe classical pathway depletion.

That SOL was possible in these sera was indicated by the partial redissolution of aggregated complexes in the IIP assay, and this was confirmed in the SOL assay: whereas SOL was in the normal range for all 15 sera at 2 h, in most cases this SOL was preceded by a prolonged lag phase compared with normal serum. Fig. 1 *B* shows the results obtained with the five sera of patients with acquired depletion of classical pathway components (paraproteinemia and benign tumor). The four sera in which SOL was not delayed were: the two C1 inactivator-deficient sera without classical pathway depletion, C1q-deficient serum, and one serum containing cryoglobulins (patient 12).

To establish that these defects in IIP and SOL were related to absent classical pathway activity, the two assays were repeated in C1q and C4-deficient sera repleted with the missing component. IIP and SOL were restored by repleting C4-deficient serum with purified C4 (Fig. 2). In separate experiments it was possible to establish that only 1% of C4 (3 μg/ml serum) was required to normalize IIP. In addition in the IIP assay, early precipitation of complexes was faster in this serum after chelation with 10 mM EGTA plus 2 mM Mg or with EDTA. Since the macromolecular C1 complex delays immune aggregation, this observation could be due to C1 dissociation after chelation of calcium (37). Furthermore, initial aggregation was faster in C1q deficient than in C4-deficient serum, and there was no difference in the initial aggregation in C1q-deficient serum whether calcium was chelated or not (EGTA-Mg or EDTA). In C1q-deficient serum and in another serum where C1q was diminished predominantly (patient 6), IIP was restored by purified C1q: 15 μg/ml serum (20% of normal) was necessary to normalize IIP in C1q-deficient serum. The immune complexes detected by the C1q binding assay in both sera seem not to have interfered with repletion experiments.

In sera containing cryoglobulins, defective IIP could also have been due to the cryoglobulins rather than hypocomplementemia. We obtained again fresh serum of patient 11 (mixed cryoglobulinemia IgMk-IgG) in which hemolytic C4 was 1% of normal, whereas other complement proteins were less depleted. The IIP assay was performed in the presence of absence of purified C4 (100 and 200% of hemolytic C4). In this serum, kept at 37°C, purified C4 was not consumed after an incubation of 15 min in a control experiment without immune complexes, whereas 80% was consumed when complexes were added. Thus

Table I. Hypocomplementemic Sera

Patients and diagnosis	Functional complement assays (% NHP)* CH50	AP50‡	Concentrations of reduced complement proteins (% NHP)§
Early classical pathway component defects			
1 C1q deficient/SLE-like disease	0‖	85	0 C1q
2 C4 deficient/SLE-like disease	0‖	100	0 C4
Acquired depletion of classical pathway components/associated with:			
3 Uterine myoma and angioedema	0	80	7% C1q, 35% C1-In, <1% C4, 10% C2
4 Paraproteinemia	0	100	10% C1q, 50% C2
5 Paraproteinemia	0	100	15% C1q, 11% C1-In, <1% C4, 25% C2
6 Paraproteinemia	0	120	13% C1q, 17% C1-In, <1% C4, 25% C2
7 Paraproteinemia	0	100	24% C1q, 21% C1-In, 5% C4, 32% C2
Cryoglobulinemia:			
8 Type I IgG lambda/vasculitis-arthritis	0	100	40% C1q, 5% C4
9 Type II IgA lambda-IgG/vasculitis	0	100	45% C1q, 7% C4
10 Type II IgM kappa-IgG/vasculitis-nephritis	0	85	35% C1q, 16% C4, 46% C2
11 Type II IgM kappa-IgG/vasculitis-nephritis	20	90	33% C1q, 5% C4
12 Type III IgM-IgG/vasculitis	50	100	15% C1q, 10% C4
C1 inactivator deficient/angiodema:			
13 Treated with danazol	100	100	39% C1-In
14 Treated with stanazol	73	63	70% C1-In
15 Treated with tranexamic acid	0	73	15% C1-In, 20% C4**, 29% C2
C3 depletion and P deficient			
16 Nephritic factor/glomerulonephritis	25	0	10% C3
17 Nephritic factor/glomerulonephritis	35	0	5% C3
18 Nephritic factor/glomerulonephritis	25	0	10% C3
19 Poststreptococcal glomerulonephritis	15	0	15% C3
20 P deficient/healthy	69	50‖¶	0 Properdin
Membrane attack complex deficiencies			
21 C7 deficient/one episode of meningitis (Neisseria meningitis)	0	0	0 C7
22 C7 deficient/one episode of meningitis (Neisseria meningitis)	0	0	0 C7
23 C8 deficient/recurrent upper respiratory tract infections	0	0	0 C8

* NHP, normal human serum pool from 25 controls. ‡ AP50 lytic alternative pathway. § Only values below the normal range are mentioned. ‖ Restored to normal with repletion of the deficient complement component. ¶ Partial lysis. ** But <1% functional hemolytic C4.

C4 function was restored, and this C4 was activated by BSA-anti-BSA complexes. However, IIP was only partially improved; after an incubation of 15 min, the percentage of soluble complexes was 96% in NHS, 70% in the cryoglobulinemic serum, and 75 and 76%, respectively, after repletion with the two concentrations of C4. Since the CH_{50} of the repleted sera was only 50% of normal, it is clear that complement function has not been fully restored by C4. Thus, hypocomplementemia (here C4 depletion) is at least in part responsible for detective IIP; however, cryoglobulins by themselves could have an additional detrimental role on IIP.

IIP and SOL assays in sera with profound C3 depletion, and in P-deficient serum. SOL was abolished in all sera. By contrast, IIP was only defective: the aggregation of complexes proceeded at a very reduced rate; i.e., >85% were still soluble after 30 min (Fig. 3). P-deficient serum showed the widest difference between the two assays (Fig. 4): a completely preserved IIP contrasted with no significant SOL over a period of incubation of 2 h. In IIP experiments, addition of EGTA plus Mg so as to block classical pathway activity resulted in fast and irreversible precipitation; the second phase of partial dissolution was observed only when the P-deficient serum was repleted (100% of a normal human pool) with purified P. In repleted serum, SOL was restored as well and the presence of EGTA plus Mg only delayed the reaction. In separate experiments, SOL was shown to be directly proportional to the P concentration, thus contrasting with the role of C4 and C1q in IIP. In addition, there was no delay in SOL using low P concentrations (down to 25% of normal). To determine whether by lowering the immune complex load the P-deficient serum would be capable of producing some SOL, $^1/_{10}$ the usual immune precipitate was used (0.01 μg ^{125}I-BSA plus 0.18 μg anti-BSA antibody per assay): there was still only a marginal 8% SOL compared with the 5% in the control P-deficient serum incubated with 10 mM EDTA.

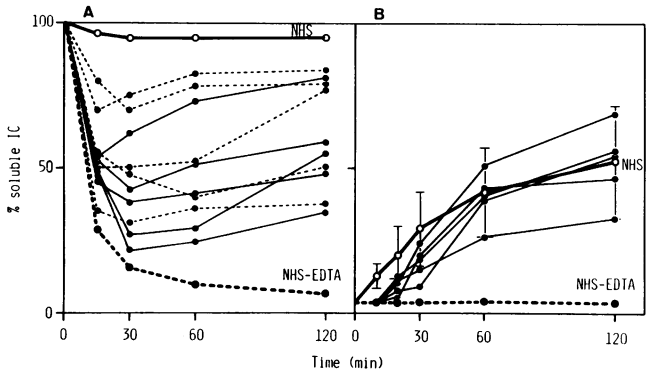

Figure 1. (*A*) IIP of ^{125}I-BSA–anti-BSA antibody complexes in various sera. The initial reaction mixture containing 0.5 μg ^{125}I-BSA and 50 ml serum was diluted to a final volume of 120 μl with CFD buffer, and incubated for 5 min at 37°C. 9 mg of rabbit anti-BSA antibody was added and the incubation was continued at 37°C. At various time intervals, 25-μl aliquots were removed, mixed with 1 ml of cold PBS buffer, and centrifuged for 15 min at 3,000 *g*. After separation of pellet and supernatant, the percentage of soluble complexes was determined. NHS: (— o —); NHS chelated with 10 mM EDTA (NHS-EDTA): (--- ● ---). Patients of group *A* of the table: patients 3–7 (uterine myoma, paraproteinemia): (— ● —) patients 8–12 (cryoglobulinemia): (--- ● ---). (*B*) SOL of ^{125}I-BSA–anti-BSA aggregates in different sera. Preformed immune aggregates (1.9 μg) were incubated at 37°C with 50 μl test serum in a final volume of 120 μl. SOL±2 SD in normal human serum are indicated: (— o —). Patients with early classical pathway depletion (patients 3–7): (— ● —).

IIP and SOL in membrane attack complex protein def. IIP and SOL were normal in sera with C7 or C8 def.

Relationship between immune complex size and abnormalities in IIP and SOL. To see whether the differences in IIP and SOL obtained in the various sera could be related to the size of the soluble immune complexes formed, the experiments were repeated and the size of the complexes determined by SDGU after an incubation of 2 h. In normal human serum, IIP and SOL produced complexes of similar size; however, since SOL was not complete (normal: 55%), a fraction of the initial insoluble complexes was recovered in the pellets of the ultracentrifuged tubes (Fig. 5).

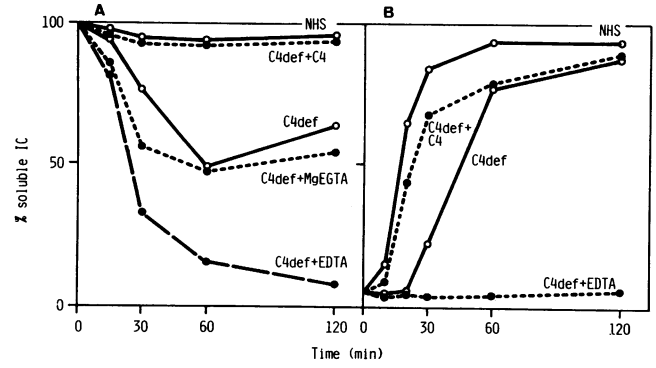

Figure 2. IIP (*A*) and SOL (*B*) in C4-deficient (def) serum. C4 def: C4-def serum; +C4: repleted with 300 μg/ml serum of C4; +EDTA: chelated with EDTA to block all complement function; +Mg-EGTA: chelated with 2 mM Mg + 10 mM EGTA to block only classical pathway activity.

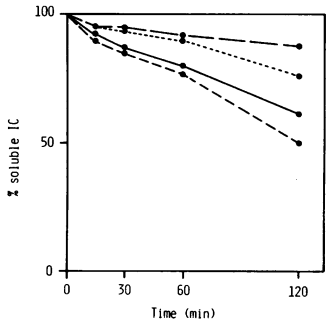

Figure 3. IIP in the four sera of patients with C3 depletion (patients 16–19). Patients: 16: — ● —; 17: - - - ● - - -; 18: — — ● — —; 19: --- ● ---.

In patients with early classical pathway depletion or def (group a), the size of the soluble complexes formed was normal in both assays. However, in IIP experiments a fraction of the complexes was recovered in the pellets. This fraction corresponded well to the fraction of insoluble complexes determined by centrifugation at 3,000 *g* in the IIP assay. After repletion of C4 and C1q in the corresponding deficient sera, no pelleted insoluble complexes were formed. Thus classical pathway defects affected only the proportion of the soluble immune complexes formed but not their size.

In contrast, in the four C3-depleted sera not sustaining any SOL (group b), the complexes formed during IIP were larger than in normal human serum in two, did not form a homogeneous population in one, and aggregated in one (Fig. 6). The soluble complexes formed in P-deficient serum were larger than those formed in normal serum; however, their size was normalized by repletion with P (Fig. 7). Thus, alternative pathway dysfunction abolished the formation of normal size soluble complexes. In membrane attack complex def (group c), the size of the soluble complexes was not modified.

Discussion

Several lines of evidence suggest that complement is involved in the elimination of immune complexes. Firstly, complement-deficient states are associated with various types of immune complex diseases (1). Secondly, complement affects directly the structure of immune complexes either by breaking up insoluble immune aggregates or by maintaining immune complexes soluble at the time of the antigen-antibody reaction (2, 3). In this study we compared IIP and SOL—the two complement functions that bring about the formation of soluble complexes—in various hypocomplementemic states to see whether defects in

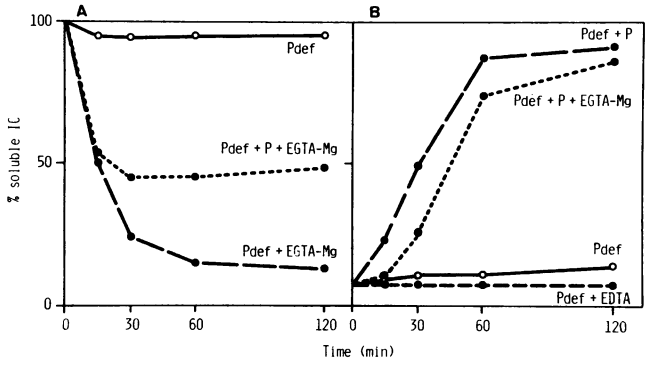

Figure 4. IIP (*A*) and SOL (*B*) in P-deficient (def) serum.

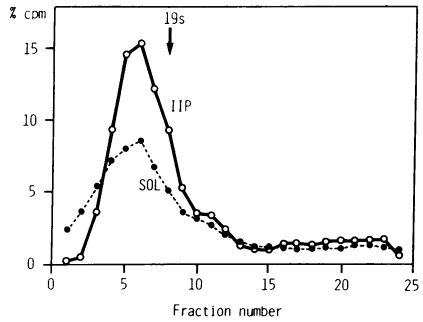

Figure 5. SDGU of soluble immune complexes formed by IIP and SOL after an incubation of 2 h in NHS. 24 fractions were collected, and results were expressed in percentage of total cpm recovered. Bottom of the gradient is on the left. IgM served as a 19-S (Svedberg unit) marker. The percentage of complexes recovered in the pellets were 4% for IIP and 25% for SOL.

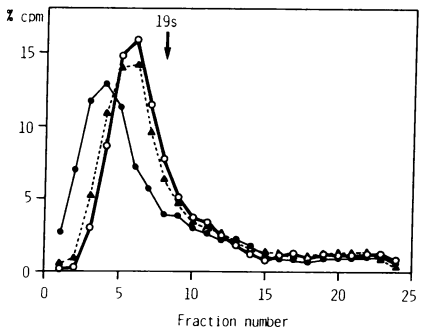

Figure 7. SDGU of complexes formed in P-deficient serum repleted or not repleted with P. The percentage of complexes recovered in the pellets were: P deficient: 13% (— ● —); P deficient + P: 8% (- - - ▲ - - -); normal serum: 8% (— o —). s, Svedberg unit.

these two functions could explain the association of complement deficiency and immune complex disease. IIP and SOL were not affected similarly in the various sera tested. Abnormalities in IIP were related to early classical pathway defects, whereas SOL was abolished in the absence of AP function.

In C1q and in C4 deficiency, IIP was severely defective mainly in its initial phase; however, immune aggregation was followed by partial redissolution of the complexes, suggesting that the SOL process was intact. This was confirmed in the SOL assay which was only preceded by a prolonged lag phase in C4-deficient serum. That defective classical pathway function was responsible for the incapacity to prevent rapid immune aggregation was demonstrated by repleting the two deficient sera with their respective missing component.

It has been shown that C4 fragments, despite binding to complexes, have little or no capacity to delay immune aggregation (37, 38). The finding that only 1% hemolytical C4 was sufficient to restore IIP in the C4-deficient serum suggests that the main role of C4 is to provide an initial C3 convertase attached to the complex so as to obtain immediate AP activation. The relative role of C4 in IIP was also evident in the three patients

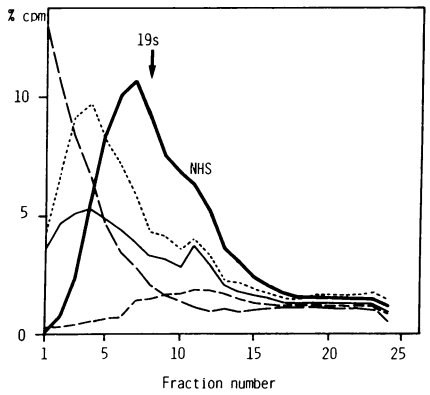

Figure 6. SDGU of complexes formed during IIP after an incubation of 2 h in the four sera with C3 depletion (patients 16–19). The percentage of complexes recovered in the pellets were: NHS: 0.5%; patient 16: 34% (—); 17: 68% (- - - -); 18: 29% (— — —); 19: 11% (----). s, Svedberg unit.

with C1 inactivator deficiency, since only the patient with profound C4 depletion presented an initial defect in IIP.

Macromolecular C1 has a direct inhibitory effect on immune aggregation at the time of the antigen-antibody reaction, and this effect is proportional to its concentration (37). Thus, C1q incorporated in the C1 complex does not only initiate classical pathway activation but directly prevents the immediate aggregation reaction. This C1 effect was probably present in C4-deficient serum, since initial immune aggregation was not as fast as in C1q-deficient serum.

In P-deficient serum, IIP and SOL were affected very differently. The essential role of Properdin in SOL is well established; however, we wanted to know if a serum deficient in only this component, and in the face of a reduced immune complex load, whether some SOL would be possible. The results were clear: no significant SOL occurred in any of the experiments done using our immune complex model. This SOL was restored by purified P and was directly proportional to P concentration. The absent SOL contrasted with the capacity of this serum to prevent immune precipitation, confirming results obtained with a serum depleted of Properdin (6). Complement mediated IIP, like SOL, did not require the assembly of the membrane attack complex (C5b to C9), since IIP and SOL were normal in C7 and C8 deficient sera.

In sera of patients with complement-depleted states, alterations in IIP and SOL could be directly related to missing complement function or proteins: early classical pathway depletion correlated with initial defects in IIP and C3 depletion with abolition of SOL. In the later group, IIP was not as much preserved as in P deficiency: aggregation of complexes could not be prevented completely, but did proceed at a very reduced rate. A similar slow aggregation has been reported previously in serum depleted of C3 with cobra venom factor and in C3-deficient serum (3). Thus, despite the absence of C3 there was still a considerable delay in immune aggregation in such sera, contrasting with the rapid aggregation observed in early classical pathway component deficiencies or depletion.

Polyclonal and monoclonal IgM rheumatoid factors have been reported to block normal IIP (21, 22); it is possible that this effect was present in some patients with classical pathway depletion and cryoglobulinemia types II and III. However, hypocomplementemia was involved in the defective IIP as well, since repletion of C4 in one such serum restored IIP in part.

Immune elimination of complexes in vivo has been shown

to be proportional to their size, large complexes disappearing first (39). To see whether the soluble complexes formed by IIP and SOL in various sera were of similar size, we analyzed them by SDGU. In normal serum, IIP and SOL produced large soluble complexes (>19 S) of identical size. In pathological sera, the proportion of soluble complexes becoming or remaining soluble was reduced, as already shown in the IIP and SOL assays; however, the size of the soluble complexes formed remained unchanged as long as alternative pathway function was preserved. Only sera with C3 depletion and P-deficient serum, those which did not sustain any SOL, produced soluble complexes of larger size during IIP. These results suggest that under normal circumstances all soluble complexes formed either by IIP and by SOL have been processed by the AP. Thus, SOL of preformed aggregates produces only one type of complexes; i.e., AP processed. In contrast, at the time of the antigen-antibody reaction, complement can maintain complexes soluble by different successive mechanisms: (*a*) C1 itself protects complexes from aggregation for a short period of time (6); (*b*) classical pathway activation is capable of producing large complexes of reduced precipitability; and (*c*) AP action (including P) reduces the size of such complexes. No in vivo experiment have been done yet to see whether such differences in size of large complement reacted soluble complexes (>19 S) influence their elimination.

In vivo it is likely that complement is continuously involved in the formation of soluble complexes so that the reaction between antigen and antibody does not lead to the formation of insoluble material. Prevention of immune aggregation could allow complexes formed in the extravascular space to diffuse into the lymphatic and blood vessels, and those formed in the blood stream not to deposit in tissues. Furthermore, it has been suggested that C3 binding to the complexes is of major importance for the safe disposal of large soluble complexes (40). Indeed, in primates, whose erythrocytes have C3b receptors, such complexes have been shown to be delivered to the fixed macrophage system on the surface of erythrocytes.

Clinical indications on the relevance of complement-mediated IIP comes from the strong association between different early classical pathway component deficiencies and immune complex diseases (1). The C1q- and C4-deficient patients investigated here are no exceptions; most such patients suffer from SLE-like diseases. Interestingly, C3 deficiency is less often associated with immune complex disease than are C1q and C4 deficiencies. The partial delay in immune aggregation could still be protective to some extent. Of particular interest is the observation that IIP is preserved in P-deficient serum: none of the seven patients described to date has an immune complex disease (29, and Sjöholm, A., and P. Spaeth, unpublished observation). This casts some doubts on the possible protective role of SOL, since SOL is abolished in the absence of P. It is therefore likely that in many situations, normal classical pathway function is essential and sufficient to protect the organism against the harmful effects of immune complex formation and deposition in various tissues.

The consequences of acquired defects of complement components are likely to be similar to inherited deficiencies, since comparable defects in IIP are observed. Nephritic factor and profound C3 depletion are associated with membranoproliferative glomerulonephritis type II; however, such patients have no vasculitis. Furthermore, deposits in the glomeruli contain most often C3 fragments but little or no immunoglobulin (41). Thus, there is no evidence that this disease is mediated by immune complex deposition. In contrast, generalized vasculitis and arthritis are prominent in cryoglobulinemia and suggest a disease process similar to acute serum sickness in which vasculitic lesions are due to immune complex deposition from the blood stream. Thus, it could well be that in many pathological situations (cryoglobulinemia, SLE, bacterial endocarditis), the depletion of the classical pathway of complement contributes to the formation of insoluble complexes.

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