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Solubilization of immune precipitates by complement in the absence of properdin or factor D

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Various experiments have demonstrated that immune precipitates (IPs) are not solubilized by complement in the absence of alternative pathway function. To determine whether the characteristics of the IPs were responsible for these observations, we studied the solubilization (Sol) of IPs formed by bovine serum albumin (BSA)-rabbit antiBSA and tetanus toxoid (TT)-human antiTT. Sera deficient in properdin solubilized a fraction of BSA-antiBSA precipitates, although only when the IPs were formed in antibody excess. The same sera solubilized TT-antiTT precipitates with some delay but almost as efficiently as normal serum. Factor D-depleted serum solubilized a fraction of TT-antiTT precipitates too, indicating that Sol may proceed through activation of the classical pathway only. Thus, the requirements for complement-mediated Sol depend on the characteristics of the IPs and do not necessarily include alternative pathway function.

Solubilization; Immune complex; Complement; Properdin; Factor D

1. INTRODUCTION

In 1975, Miller and Nussenzweig [1] reported that complement solubilizes immune precipitates (IPs). Multiple studies have demonstrated that the covalent binding of C3b to antigen and antibody molecules was responsible for the fragmentation of the lattice [2–4]. Solubilization (Sol) was shown to be dependent on both pathways of complement, alternative pathway activation being essential for Sol, and the classical pathway allowing a rapid initiation of the process, i.e. the initial C3b deposition [1,5–7]. Significant solubilization did not occur in the absence of C3, Factor B, Factor D or Properdin [7–11]. However Sol differs widely from one type of IP to another, depending on the antigen/antibody ratio used to form the IP and the characteristics of the antigens and the antibodies [2,12,13].

For these reasons we reinvestigated the Sol of different types of IPs in reagents with defective or absent alternative pathway function, i.e. in properdin-deficient sera (Pdef), and in serum that had been immunochemically depleted of Factor D (RD).

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2. MATERIALS AND METHODS

2.1. *Immune precipitates*

Bovine serum albumin (BSA) (Calbiochem) was radiolabelled with ^{125}I by the lactoperoxidase method (Calbiochem). Precipitating rabbit antiBSA antibodies (Dako) were depleted of cross-reactive anti-human serum albumin (HSA) antibodies by adsorption with glutaraldehyde cross-linked HSA (Swiss Red Cross). After determining the equivalence point, IPs were formed at different antibody/antigen ratios from equivalence to 10-fold antibody excess. After centrifugation (3000 × *g* for 15 min) the precipitates were suspended in phosphate buffered saline (PBS, Oxoid). The concentration of IPs was determined by the Folin method.

Radiolabelled tetanus toxoid (TT)-human antiTT IPs were prepared at equivalence [14].

2.2. *Sol and immune assays*

The Sol assay was performed as described [11]. Briefly, IPs were added to 100 μl of serum in a final volume of 200 μl using PBS as a diluent. The mixtures were incubated at 37°C and aliquots (40 μl) were removed at various intervals and mixed with

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1 ml cold PBS. The percentage soluble radioactivity was determined after centrifugation at 3000 × g for 15 min. The Sol of BSA-rabbit antiBSA precipitates, formed at equivalence, was studied using various concentrations of IPs, from 13 μg/ml to 130 μg/ml (relative concentration of IPs to ml of serum). Sol of IPs at 4- and 10-fold antibody excess was performed with IP concentrations of 40 μg/ml and 24 μg/ml, respectively. The concentration of TT-human antiTT precipitates was 10 μg/ml of human serum. Adherence of solubilized TT-antiTT complexes to human erythrocytes was determined using the fraction of soluble complexes obtained after centrifugation [15].

2.3. *Sera and purified proteins*

Fresh normal sera were obtained from healthy donors and stored in liquid nitrogen. The sera of patients with properdin deficiency (Pdef) [16], Factor I deficiency [17], and C1q deficient serum (C1q def) (kindly provided by M. Walport) were stored at −70°C before use.

Normal serum depleted of Factor D (RD) was prepared by affinity chromatography using rabbit polyclonal antiFactor D antibodies. Rabbits were immunized with purified Factor D (kindly provided by J. Volanakis) three times in complete Freund's adjuvant at biweekly intervals. Two weeks following the last immunization, the serum was shown to contain antibodies against Factor D using a functional plate assay for Factor D [18]. The IgG fraction of this antiserum was bound to cyanogen bromide-activated Sepharose (Pharmacia). The antiFactor D affinity-chromatography step removed all functional Factor D (limit of sensitivity for the haemolytic assay: 0.1% of normal Factor D concentration, i.e. more than 99.9% of Factor D was removed). The RD was concentrated to 2/3 of its original volume and stored in liquid nitrogen until usage. The haemolytic activity of RD could be restored to normal (as compared to the initial serum) with purified D in a functional alternative pathway plate assay [18]. In the Sol experiments, RD was compared to its initial serum. C1q def serum was depleted of Factor D (RD/C1q def) in the same way. Sera devoid of C3 function were obtained by depleting C3 with cobra venom factor [15]. Classical pathway function was blocked by adding 10 mM ethyleneglycol tetraacetate and 2 mM magnesium (MgEGTA).

Properdin was purified according to Medicus et al. [19].

The concentration of antiTT antibodies in human sera was measured by a standard ELISA technique, the antihuman IgG being a peroxidase-conjugated rabbit IgG (Pasteur); the concentrations measured were compared to that of the antiTT IgG (Berna) used to form the IPs. The RD was prepared from a serum that contained no antiTT antibodies.

3. RESULTS

When formed at equivalence, BSA-rabbit anti-BSA IPs were not solubilized by 4 different Pdef sera even after an incubation of 120 min; in normal human serum Sol increased from 8 ± 2.5% to 86 ± 13% using decreasing concentrations of IPs (from 130 μg/ml to 13 μg/ml), whereas Sol was about 4% in Pdef sera at all IP concentrations. However, when the IPs were prepared in antibody excess, significant Sol could be demonstrated in Pdef sera (fig.1), although the percentage of IPs solubilized never reached the normal range. When the classical pathway activation was blocked with MgEGTA, Pdef sera lost their capacity to solubilize IPs formed in antibody excess (Sol ≤ 4%; normal range 30 ± 10% and 50 ± 20% for IPs formed at 4- and 10-fold antibody excess, respectively). Two different Factor I deficient sera, i.e. depleted in C3, were unable to sustain any Sol. These observations, suggesting that Sol could take place despite the absence of properdin, led us to study the Sol of IPs made with human antibodies, i.e. TT-antiTT precipitates.

When Sol of the two types of IPs formed at equivalence was compared, striking differences became evident (fig.2). TT-human antiTT IPs were solubilized in the absence of properdin, and although the reaction was slightly delayed, the percentage solubilized at 120 min was in the normal range. Purified properdin restored the normal rate of Sol. Three different properdin deficient sera were tested, and similar results were obtained. Sol was not due to the presence of antiTT antibodies in the Pdef sera: the titer of antiTT antibodies was less or equal to that used to form the IPs, i.e. the reaction occurred in no more than twofold antibody excess, a concentration of an-

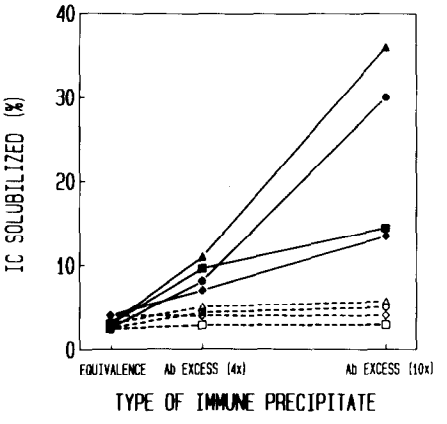

Fig.1. Solubilization of BSA-antiBSA precipitates by 4 different Pdef sera. The percentage of soluble immune complexes (ICs) in the Pdef sera (closed symbols) and in the same sera in the presence of 10 mM EDTA (open symbols) was determined after an incubation of 120 min at 37°C. Sol of IPs formed at 4-fold and 10-fold antibody excess in normal serum: 64 ± 20%, and 90 ± 10%, respectively.

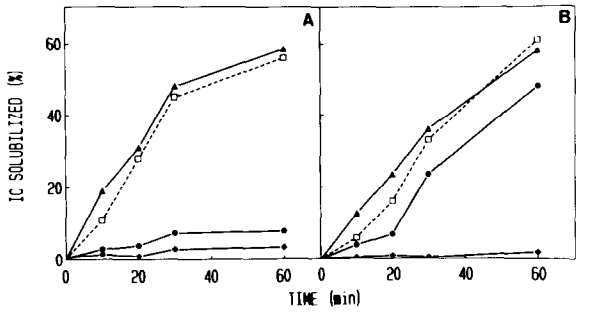

Fig.2. Solubilization of immune precipitates formed at equivalence: (A) Sol of BSA-rabbit antiBSA (13 μg/ml), and (B) of TT-human antiTT (10 μg/ml) by NHS (□---□), by Pdef serum (●—●), by Pdef serum repleted with P (2 μg/ml) (▲—▲), and by Pdef serum in the presence of MgEGTA (◆—◆). In the presence of 10 mM EDTA, no Sol was observed in either system.

tibodies that did not produce any dissociation of IPs (24 h at room temperature in PBS) and that did not modify Sol in normal human serum (not shown).

The incorporation of C3b into immune complexes (ICs) solubilized by Pdef sera was demonstrated by immune adherence. The ICs solubilized after 30 min were exposed to human erythrocytes: 31% of them bound to the erythrocyte C3b receptors as compared to 43% of those solubilized by normal serum (≤2% binding to erythrocytes that have been stripped of their C3b receptors by trypsin treatment) [15].

Table 1

Solubilization of preformed TT-antiTT immune precipitates by different reagents after an incubation of 120 min at 37°C

Reagent	Reagent added	TT-antiTT IP solubilized (%)
NHS[a]	–	95
NHS	10 mM EDTA	11
RD	–	40
RD	6% Factor D[b]	47
RD	30% Factor D	76
RD	100% Factor D	89
RD	MgEGTA	12
C1q def/RD	–	11
C1q def	–	70
C3 depleted	–	10
Buffer	–	12

[a] Used to obtain RD

[b] 100% of Factor D added corresponds to 2 μg/ml

RD was able to promote Sol of TT-human antiTT IPs, although this Sol was reduced (table 1). Purified D increased Sol in a dose dependent manner. The addition of MgEGTA to RD abolished Sol, indicating that Sol was probably due to the classical pathway activation; this was demonstrated by depleting Factor D in a C1q def serum: the RD/C1q def reagent did not mediate any Sol. RD did not solubilize BSA-antiBSA IPs.

4. DISCUSSION

The results obtained in this work demonstrate that some types of immune precipitates can be solubilized by activation of the classical pathway of complement only, with no need for alternative pathway function.

The Sol reaction is highly dependent on the antigen and the antibodies used to form the precipitates, and the antigen-antibody ratio determines the extent of Sol as well [13]. The initial observation of some Sol of rabbit IPs formed in antibody excess in human serum deficient in properdin, led us to question the general statement about the requirement for an alternative pathway (AP) function. Thus, we further investigated Sol by human serum of IPs made with human antibodies. Our results show, that the Sol of TT-antiTT does not require properdin. Furthermore complete blockade of AP function, as obtained by depleting Factor D, did not abolish Sol.

The absence of total dependence on the AP function has already been suggested, Genin and Lesavre [20] depleted human serum of Factor B by heat inactivation at 50°C and showed that radiolabelled antiTT antibodies were released from TT-antiTT precipitates formed in antigen excess, but not from those formed in equivalence or antibody excess.

The results presented here do not contradict previous observations. The mechanism of complement activation responsible for Sol may be very different from one IP to another. In addition to the characteristics of the antigen and the antigen-antibody bonds, the Fc-Fc interactions are likely to participate to the structure of IPs formed in antibody excess. TT-human antiTT precipitates formed at equivalence might also be held together, in part, by Fc-Fc interactions. The rapid initial precipitation of antigen-antibody complexes form-

ed at equivalence has been shown to be due to Fc-Fc interactions [21], and this precipitation is blocked by activation of the classical pathway of complement [9]. Thus, Sol by the classical pathway might be due to the disruption of such weak Fc-Fc interactions.

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