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Immune adherence and staphylococcus protein A binding of soluble immune complexes produced by complement activation

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SUMMARY

Complement has been shown to affect the solubility of antigen–antibody complexes by two mechanisms: in the first, classical pathway dependent, complement inhibits the formation of the immune precipitate; in the second, alternative pathway dependent, complement reacts with a formed precipitate to bring about its solubilization. The biological properties of complement reacted immune complexes (IC) has been assessed by studying their binding to staphylococcus protein A (SPA) and to human erythrocytes. BSA–anti-BSA complement reacted IC bound to human erythrocytes and to SPA. Complexes generated by solubilization of immune precipitates showed greater immune adherence than complexes held in solution by complement, despite their similar size. Complexes held in solution in a factor D depleted human serum bound more efficiently to erythrocytes than complexes formed in normal serum. These experiments demonstrate that complement reacted IC cannot be regarded as biologically inert and that factors affecting complement function may have important effects on the properties of antigen–antibody complexes.

Keywords soluble immune complexes complement activation

INTRODUCTION

The solubility of immune complexes (IC) has been shown to be modified by two recently defined complement-dependent mechanisms. (1) At the time of the reaction between antigen and antibody complement is capable of blocking immune precipitation and causes the immediate formation of small soluble complexes mainly by classical pathway activation (I-IC) (Schifferli, Bartolotti & Peters, 1980; Schifferli, Woo & Peters, 1982). (2) Immune precipitates can be solubilized by complement by a mechanism involving alternative pathway activation (S-IC) (Miller & Nussenzweig, 1975; Takahashi, Takahashi & Hirose, 1980).

Little is known about the binding reactivity of these complement reacted complexes either to complement or Fc receptors.

The few data available suggest that S-IC are 'dead end' complexes (Nussenzweig, 1980)—they seem to be immune adherence negative and their reactivity via Fc binding seems to be abrogated (Takahashi *et al.*, 1980; Scharfstein *et al.*, 1979). For instance Scharftein *et al.* (1979) demonstrated that solubilized complexes lost their binding affinity to staphylococcus protein A (SPA) and suggested that the Fc portion of the immunoglobulins of the complexes were covered by

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complement fragments. The binding reactivity of I-IC has not been studied. As such complexes are likely to be present *in vivo*, it is relevant to study and compare the binding of these two types of soluble IC to C3b receptors and to examine the availability of their Fc portions.

MATERIALS AND METHODS

IC. ^{125}I-BSA–rabbit anti-BSA Ab as immune precipitates or as soluble IC were prepared at equivalence in normal human serum, factor D depleted serum (RD) or EDTA treated serum as previously described (Schifferli *et al.*, 1982). In brief three different types of soluble IC were formed: (1) by complement-mediated solubilization of a pre-formed immune precipitate in normal human serum (NHS); (2) by complement-mediated inhibition of immune precipitation in normal human serum; (3) and in RD.

Three different types of immune precipitates were studied: (1) those formed in EDTA treated serum by immune precipitation; (2) and already formed precipitates incubated in EDTA treated serum; (3) or in RD, both reagents being incapable of producing solubilization. EDTA treated serum = 10 mM EDTA.

For some experiments IC were partially purified by sucrose density gradient ultracentrifugation (10–50% wt/wt sucrose). Forty-eight fractions were collected and the five fractions containing the highest concentrations of IC were used after dialysis against phosphate-buffered saline (PBS; Oxoid). (Peak at 25S for S-IC and I-IC formed in normal human serum, larger for those formed in RD; Schifferli & Peters, 1982).

IC attachment. The attachment of IC to human erythrocytes was measured using a modification of the method described by Takahashi *et al.* (1980). IC were incubated for various periods of time with serum: for solubilization experiments the mixtures contained 0·5 μg ^{125}I-BSA/equivalent amount of rabbit anti-BSA in 500 μl of serum; for inhibition of immune precipitation experiments, 5 μg ^{125}I-BSA/equivalent amount of antibody in 500 μl of serum. Aliquots of 100 μl of these mixtures were added after various periods of time to 300 μl of a suspension containing erythrocytes in PBS + 20 mM EDTA (to block further complement activation). The suspension contained 10% erythrocytes. In some experiments varying concentrations of erythrocytes were used (0·5–20%, or 5–25%). After 5 min incubation at 37°C in the erythrocyte suspension 100 μl aliquots were overlayered onto 1 ml Ficoll-Hypaque (Ficoll-Plaque lymphoprep, Pharmacia) and centrifuged at 120*g* for 10 min. The supernatant and Ficoll-Hypaque containing the unbound IC were gently removed with a pipette without disturbing the erythrocyte pellet. The percentage of attachment to erythrocyte was determined by measuring the radioactivity in the erythrocyte pellet and in the supernatants. Control experiments without erythrocytes were done simultaneously and all experiments were done in duplicate.

Sepharose CL 4B protein A (SPA) and Sepharose CL 4B. These were purchased from Pharmacia and small disposable immunoadsorbant columns were made using Eppendorf pipette tips supported in 2 ml plastic test tubes (LP3) as described by Pearson & Anderson (1980). The column was packed with 400 μl of 10% immunoadsorbant in PBS (SPA–Sepharose CL 4B and Sepharose CL 4B) and washed twice in PBS (final column volume = 40 μl). Serum samples containing IC were applied to the top of the column; after 15 min incubation at room temperature, these columns were washed with a total volume of 500 μl of PBS. The immunoadsorbant was dried in the last wash by centrifugation at 800*g* for 5 min. The percentage of binding to the column was determined from the radioactivity recovered in the immunoadsorbent and in the 500 μl effluent.

RESULTS

Binding of IC to erythrocytes

Binding of S-IC to different concentrations of erythrocytes. The binding of IC solubilized by NHS for 10 min (64% soluble, 38% still insoluble) was dependent on the concentration of erythrocytes used (Fig. 1): even with 2% erythrocytes there was significant binding and at 20% more than 50% of

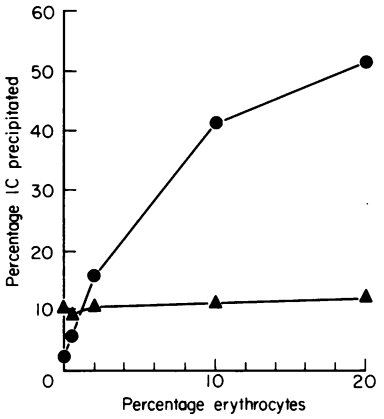

Fig. 1. Binding of IC to increasing concentrations of erythrocytes. ●—● = immune aggregates solubilized for 10 min by complement; ▲—▲ = immune aggregates incubated in EDTA serum. Note the increasing percentage of binding of IC exposed to normal human serum (NHS) and the absence of binding of IC incubated in EDTA serum.

IC were attached to erythrocytes; this degree of binding indicates that some soluble IC must have bound, as the non-solubilized IC fraction was only 38%. The same IC incubated in EDTA-NHS for 10 min (95% insoluble) did not bind to erythrocytes. The higher background precipitation with insoluble IC was caused by very large aggregates coming through the Ficoll-Hypaque at 120*g* in the absence of erythrocytes (8–10% precipitation for insoluble aggregates compared to only 1–3% precipitation for the solubilized complexes).

This background did not increase with increasing erythrocyte concentration (i.e. no non-specific trapping).

Change in binding of S-IC to erythrocytes after prolonged incubation. IC were solubilized by incubation in NHS for 10 min at which time 20 mM EDTA were added and the incubation carried on for a further 60 min. The binding dropped from 52% to 26% after incubation in EDTA using 20% erythrocytes, and from 43% to 22% using 10% erythrocytes. Since no further solubilization

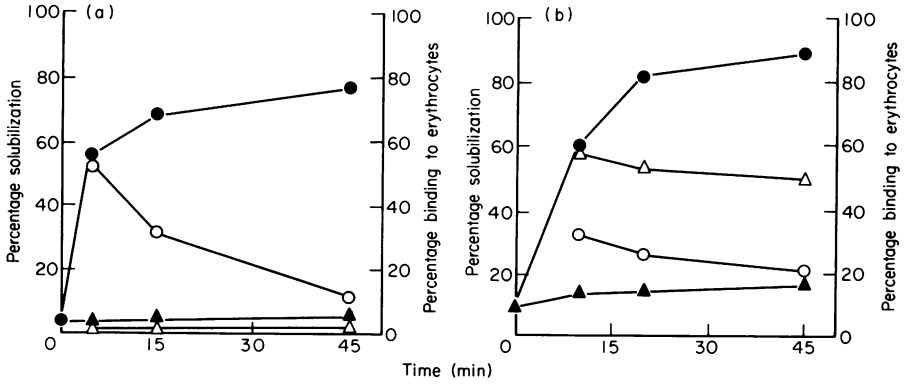

Fig. 2. (a) Kinetics of red blood cell binding of S-IC. The percentage binding is calculated after subtraction of the background precipitation without erythrocytes. Solubilization in NHS ●—●, in EDTA-NHS ▲—▲, erythrocyte binding in NHS ○—○, in EDTA-NHS △—△; in NHS, fast initial solubilization was associated with high immune adherence, which decreased with time. (b) Binding of immune aggregate incubated in a reagent depleted of factor D (RD). Solubilization in NHS ●—●, in RD ▲—▲; erythrocyte binding in NHS ○—○, in RD △—△. Note the absence of solubilization in RD and the comparatively high immune adherence of these insoluble IC.

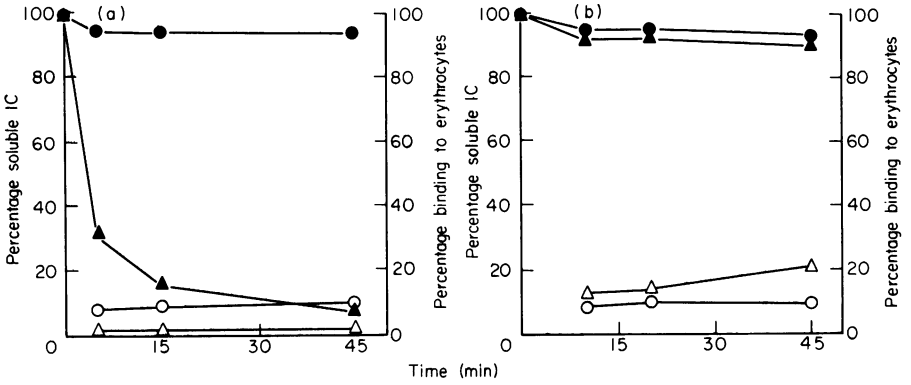

Fig. 3. (a) Kinetics of red blood cell binding of I-IC (background subtracted). Inhibition of immune precipitation in NHS ●—●, in EDTA-NHS ▲—▲; erythrocyte binding in NHS ○—○, in EDTA-NHS △—△; in NSH all IC remained in solution; a small but significant fraction of these IC showed immune adherence. Note the fast precipitation and the absence of immune adherence in the presence of EDTA. (b) Binding of I-IC formed in factor D depleted serum RD. Inhibition of immune precipitation in NHS ●—●, in RD ▲—▲; erythrocyte binding in NHS ○—○, in RD △—△. Note the absence of precipitation in RD and the small but significant higher immune adherence of I-IC formed in RD.

occurred in the presence of EDTA, it seems likely that the reduced binding of IC to erythrocytes was due to inactivation of C3b and C4b by factor I.

Time course of binding of S-IC to erythrocytes (Fig. 2a). During solubilization brought about by NHS, the binding was maximal at 5 min and diminished thereafter. In the presence of EDTA serum there was no binding of IC to erythrocytes and no solubilization took place.

In another experiment insoluble immune aggregates were incubated in RD (Fig. 2b); under these conditions no solubilization took place but a larger fraction of the complexes became immune adherence positive. Binding of these complexes to erythrocytes was greater than that of S-IC. However, like S-IC their immune adherence diminished slowly with increasing periods of incubation.

Binding of I-IC (Fig. 3a). The soluble I-IC formed in the presence of serum bound to a small but significant extent to erythrocytes. In the presence of EDTA serum, precipitation of IC occurred rapidly. These complexes did not show immune adherence.

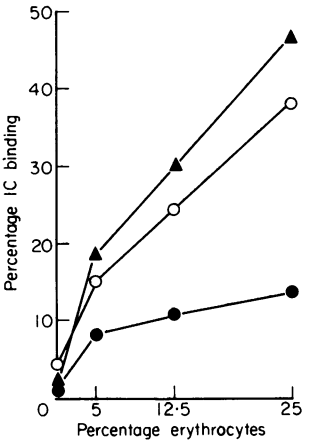

Fig. 4. Binding of partially purified soluble IC to increasing concentrations of erythrocytes. ●—● = I-IC formed in NHS; ○—○ = I-IC formed in RD; ▲—▲ = S-IC formed in NHS. Note that at all concentrations studied S-IC presented the highest immune adherence and I-IC formed in RD bound more efficiently than those formed in NHS.

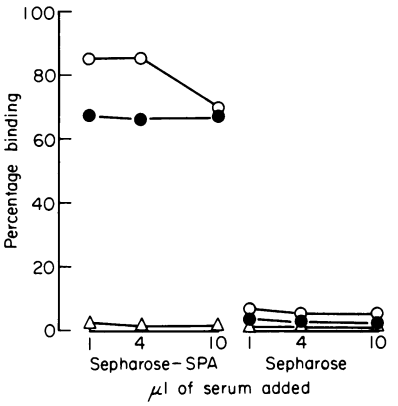

Fig. 5. Binding of I-IC to Sepharose–SPA and to Sepharose only. Insoluble IC formed in EDTA serum (90% insoluble complexes) O—O; soluble IC formed in NHS (96% soluble complexes) ●—● and ^{125}I-BSA △—△. Note the binding of soluble and insoluble IC to Sepharose–SPA only; the three different amounts of serum added to the microimmunoadsorbant gave virtually identical results.

In the absence of factor D (RD) the immune adherence of such complexes was slightly greater than that of I-IC formed in the presence of normal serum (Fig. 3b).

No significant change in immune adherence was observed during the 45 min of these experiments.

Binding of partially purified soluble IC. S-IC and the I-IC formed in NHS and in RD were partially purified by sucrose density gradient ultracentrifugation. As an identical ratio of Ag to Ab was used for all experiments, S-IC were of a similar size to I-IC formed in NHS, both these types of IC being smaller than I-IC formed in RD. The relatively stable size of these IC was demonstrated by a second ultracentrifugation in which no significant aggregation was seen. The binding of these IC is shown in Fig. 4. At all erythrocyte concentrations S-IC had the highest immune adherence. I-IC formed in the absence of alternative pathway function (RD) bound more strongly to erythrocytes than I-IC prepared in NHS.

Binding of IC to Sepharose–SPA

I-IC bound efficiently to Sepharose–SPA; the binding was similar to that seen with insoluble IC formed in EDTA serum (Fig. 5). This result was unaffected by varying the amounts of serum added to the column (1, 10 and 40 μl). ^{125}I-BSA only did not bind to the immunoadsorbent and no IC bound to Sepharose without SPA. By double immunodiffusion no IgG could be detected from the effluent of the Sepharose–SPA column loaded with 10 and 40 μl of serum, whereas IgG was present in the effluent of the control Sepharose.

Table 1. Binding of S-IC to Sepharose–SPA

	Sepharose*	Sepharose–SPA*
IC solubilized by NHS (total)†	7·9	65·3
IC solubilized by NHS (soluble fraction only)	3·2	64·8
IC incubated in EDTA-NHS	33·1	68·0

* % binding to column, serum samples of 10 μl.

† Total after 1 h incubation: soluble = 80%; insoluble = 20%.

S-IC fixed also to the Sepharose–SPA (Table 1) and this binding was no less than that of larger insoluble IC incubated in EDTA treated serum (which showed a higher background binding to Sepharose—probably because their size did not allow normal flow through the column).

The two different types of soluble IC bound to a similar extent to SPA (compare Fig. 5 and Table 1).

DISCUSSION

These experiments demonstrate that soluble IC generated by complement activation have binding affinity for both SPA (Fc binding) and human erythrocytes (C3b binding). Furthermore the binding affinity of IC to red blood cells was stronger for solubilized complexes than for those produced during inhibition of immune precipitation. These main findings merit further analysis.

Binding to erythrocytes. This was observed for all types of IC incubated in the presence of complement. Cornacoff, Zager & Herbert (1981) demonstrated that the efficiency of binding of IC preincubated with complement is mainly dependent on the size of the IC. The experiments reported here accord with this: large soluble I-IC formed in RD bound more efficiently than the smaller ones formed in NHS. Similarly large insoluble immune aggregates incubated in RD showed stronger binding than S-IC in normal serum.

In all experiments S-IC showed greater immune adherence than I-IC whether alternative pathway function was preserved or not. Solubilization requires massive C3b deposition by the alternative pathway and the more efficient binding of S-IC may reflect a higher number of C3b molecules per IC: this would allow either better multipoint attachment to C3b receptors on red blood cells as the number of receptors per cell is low (Fearon, 1980) or higher affinity for C3b receptors (Arnaout *et al.*, 1983).

In serum, C3b molecules bound to IC will be inactivated by I in the presence of H. Even though this process is rapid and accounts for loss of immune adherence, the C3b in S-IC cannot have been totally inactivated at the time of solubilization as has been previously suggested (Takahashi *et al.*, 1980), because a further decrease in their binding occurred after incubation in EDTA serum and immune aggregates incubated in RD showed a similar decrease in binding.

Binding to SPA. Both types of soluble IC generated by complement activation were retained in the SPA immunoadsorbant column; the retention was similar to that seen for immune precipitates not reacted with complement. These results are in apparent contradiction with a report of substantially reduced binding of solubilized IC to SPA (Scharfstein *et al.*, 1979). McDougal *et al.* (1979) have demonstrated that, whereas the binding affinity of SPA for IgG is constant, complexed IgG is favoured over monomeric IgG because of its capacity for multipoint attachment. Complexes formed in high antibody excess and very large complexes will thus fix to SPA more readily than monomeric IgG and be exchanged at a much reduced rate (McDougal *et al.*, 1979; Kessler, 1975). Small soluble IC can be detected in pathological sera only if monomeric IgG is first removed (Hällgren & Wide, 1976); this is probably because they contain too few IgG molecules per complex to allow privileged binding over monomeric IgG. Since the experiments of Scharfstein and co-workers were performed in the presence of serum and therefore the presence of high concentration of monomeric IgG, their results could be explained by a critical reduction in the number of IgG molecules per complex when the large complexes are split into smaller IC during solubilization. In the experiments reported here the SPA immunoadsorbant column had the effect of removing most of the monomeric IgG avoiding competition between complexed and monomeric IgG.

These experiments have established that complement reacted immune complexes cannot be regarded as biologically inert ('dead end' complexes), and further, that there are likely to be substantial differences in the *in vivo* behaviour of I-IC and S-IC. Clarification of the mechanisms underlying the biology of these complement reacted antigen–antibody complexes should lead to better understanding of the relationship between complement and IC disease.

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