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Ability of complement to release systemic lupus erythematosus immune complexes from cell receptors

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SUMMARY

Endogenous immune complexes present in sera from 10 different patients with systemic lupus erythematosus (SLE) in an active phase were allowed to bind to Raji cells; the ability of intact complement to release the cell-bound complexes from receptors was then examined. Fresh normal human serum, or, alternatively, zymosan-pretreated serum, was added to the complex-bearing Raji cells. Immune complexes remaining bound to Raji cell receptors after increasing times at 37°C were quantitated by addition of ^{125}I-labelled antiglobulin, after removal of serum by washing. In all 10 cases, complement-dependent release was observed. In parallel control studies performed under identical conditions, immune complexes prepared *in vitro* from bovine serum albumin (BSA) and guinea-pig anti-BSA antibody were used in place of the endogenous SLE complexes. The experimental complexes were released by fresh serum, but not by zymosan-treated serum, when studied using either ^{125}I-labelled anti-guinea-pig Ig or ^{125}I-labelled complexes alone. The results suggest that intact complement can alter the immune complexes present in SLE sera and influence their interaction with receptors on lymphoid cells. The results further raise the possibility that hypocomplementaemia secondarily due to consumption of complement by immune complexes may contribute to the persistence of the complexes.

INTRODUCTION

High levels of circulating immune complexes (ICs) are frequently associated with hypocomplementaemia in patients with systemic lupus erythematosus (SLE) during periods of clinical activity (Agnello, Winchester & Kunkel, 1970; Nydegger *et al.*, 1974; Theofilopoulos, Wilson & Dixon, 1976). Hypocomplementaemia is generally viewed as a consequence of consumption of complement by ICs. How biological properties or metabolism and disposal of ICs are affected by the hypocomplementaemic state is not well understood, however.

Various studies by ourselves and others (Miller & Nussenzweig, 1974; Oger & Medof, 1980) have shown that complement influences interactions of ICs with cellular membrane receptors. Under certain conditions, complement mediates binding of ICs to complement receptors (Eden, Bianco & Nussenzweig, 1973). Under other conditions, complement solubilizes ICs and releases them from cell receptors (Miller, Saluk & Nussenzweig, 1973). In biological systems containing ICs, sufficient complement and cellular receptors, a dynamic state exists in which there is first complement-dependent binding followed by complement-dependent release. The kinetics of binding and release are dependent upon the complement concentration.

The ability of complement to release bound ICs from receptors is termed *complement-dependent*

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release activity, or CRA. CRA is mediated primarily by alternative pathway components but is enhanced by simultaneous activation of the classical pathway (Takahashi, Tack & Nussenzweig, 1977; Takahashi *et al.*, 1978). C5 and later complement components apparently do not participate. CRA involves assembly of a factor B-dependent C3 convertase on the ICs. Interaction with C3 leads to solubilization. C3 fragments bind to molecules within the ICs; there is evidence that this binding results in dissociation of primary antigen:antibody bonds (Czop & Nussenzweig, 1976). Released ICs are irreversibly altered and cannot rebind.

Complement levels, therefore, should play an important role in determining properties of ICs that are present in SLE sera, and in determining whether circulating ICs in SLE patients are associated with cells bearing receptors for them, or are free in the soluble phase.

In this study we examined the question of whether the interaction of ICs with cellular receptors in SLE patients with hypocomplementaemia is influenced by the hypocomplementaemia itself, and whether this interaction can be modifed by returning the system to a state of normocomplementaemia. Raji cells were selected to study this problem, since Raji cells possess Fc and complement receptors similar to those on normal mononuclear blood cells, and since Raji cells lack membrane-bound Ig (Theofilopoulos, Dixon & Bokisch, 1974; Theofilopoulos *et al.*, 1974) so that binding of serum ICs to cellular receptors can be quantitated.

Our results indicate (1) that interactions of both experimentally-prepared and naturally-occurring ICs with the human lymphocyte membrane are highly sensitive to variations in the complement level, and (2) that the biological properties of naturally-occurring complexes in the hypocomplementaemic state can be modified by returning complement levels to normal.

MATERIALS AND METHODS

Experimental immune complexes. Crystalline bovine serum albumin (BSA) (Miles, Kankakee, Illinois) was used as antigen. To prepare antiserum, guinea-pigs were injected in the footpads with an emulsion of BSA in Freund's complete adjuvant (1 mg BSA per animal). After 5 weeks, animals were boosted by an intraperitoneal injection of 0·5 mg BSA, and 9 days later bled under ether anaesthesia. Sera were pooled and stored at −70°C. BSA was labelled with ^{125}I using chloramine T (McConahey & Dixon, 1966) to obtain a specific activity of 2×10^6 c.p.m./μg. ^{125}I-BSA was dialysed against isotonic phosphate-buffered saline, pH 7·6 (PBS), centrifuged for 50 min at 1,200 ***g*** and stored at 4°C with 0·05% sodium azide. Equivalent proportions of pooled guinea-pig anti-BSA serum and ^{125}I-BSA were determined by quantitative precipitation assay (Maurer, 1971) with EDTA to prevent complement fixation. Precipitation of maximum amounts of both antibody and BSA (equivalence) was obtained with a proportion of 300 μg BSA to 1·0 ml antiserum.

Preliminary trials yielded the following method for preparing the experimental ICs: antiserum was heated for 30 min at 56°C to inactivate complement, centrifuged (15 min, 5,000 ***g***) immediately before use, and diluted 16-fold in RPMI 1640 medium containing 0·25% gelatin (RPMI + G). One volume of BSA (labelled or unlabelled) diluted to 17 μg/ml in RPMI + G was mixed with 4 volumes of diluted antiserum, and the mixture was incubated for 30 min at 37°C (this is a proportion of 68 μg BSA to 1·0 ml antiserum, or approximately five-fold antibody excess). An equal volume of normal human serum diluted appropriately in RPMI + G was then added to the antigen:antibody complexes as a complement source, and the mixture was further incubated for 30 min at 37°C. After reaction with complement, ICs (antigen:antibody:complement) were further diluted four-fold in cold RPMI + G. ICs prepared in this manner did not precipitate; no trace of precipitate was apparent after storage for 2 days at 4°C, followed by centrifugation (15 min, 5,000 ***g***).

Binding of immune complexes to membrane receptors. Two types of ICs were used in these studies: (1) experimental BSA:anti-BSA:complement ICs (see preparation above), prepared with either ^{125}I-labelled BSA or unlabelled BSA depending upon the protocol, and (2) naturally-occurring ICs present in SLE sera. The procedure used for studying cell membrane binding of the two types of ICs was essentially identical. Either BSA:anti-BSA ICs or SLE serum was mixed with an equal volume of Raji cell suspension (2×10^7 cells/ml); the SLE sera was thawed just before use and pre-centrifuged (10 min, 1,000 ***g***, 4°C) to remove aggregates. The IC–Raji cell mixtures were

incubated for 45 min at room temperature, with periodic swirling, to allow binding of the added ICs to membrane receptors. The mixtures were then centrifuged (10 min, 200 *g*, 4°C), and the resulting cell pellets washed twice with 3 ml cold RPMI. In the case of studies with ^{125}I-BSA ICs, pellets were counted to determine the percentage of ICs bound. For release studies, pellets were resuspended to give 2×10^7 cells/ml.

Complement-mediated release of cell-bound immune complexes. Release was measured kinetically as follows: 0·5-ml aliquots of chilled complex-coated Raji cell suspension were added to 12 × 17 mm tubes containing 0·5 ml of a two-fold dilution of chilled normal human serum (as complement source) or zymosan-treated serum (as negative control). (A two-fold dilution of normal human serum was found to give optimal results in preliminary experiments. Other complement dilutions were used in some experiments.) The tubes were then placed into a 37°C water bath and, after various intervals (generally between 0 and 40 min), 0·1-ml aliquots were transferred to tubes containing 2·5 ml ice-cold RPMI, immediately vortexed and kept on ice. After the last sample was transferred, tubes were centrifuged at 4°C. In experiments in which labelled ICs were used, the supernatants were carefully separated and both supernatants and cell pellets were counted.

In experiments in which unlabelled experimental ICs or endogenous SLE ICs were used, non-released cell-bound ICs were quantitated by addition of a radiolabelled antiglobulin. In the case of experimental BSA:anti-BSA ICs, IgG fraction of rabbit anti-guinea-pig IgG (Cappel Laboratories, Cochranville, Pennsylvania) was employed; it was labelled with ^{125}I using chloramine T to obtain a specific activity of 1×10^6 c.p.m./μg. In the case of endogenous SLE ICs, Raji cell-bound ICs were quantitated using ^{125}I-labelled monoclonal rheumatoid factor (^{125}I-mRF) (see below). Following the release procedure, the cell pellets were resuspended in 0·1 ml RPMI+G, together with 30 μg of ^{125}I-anti-guinea-pig IgG or with 1 μg ^{125}I-mRF. The mixtures were incubated for 30 min at 4°C, with periodic gentle mixing. Finally, the cell suspensions were washed twice with 2 ml RPMI+G, and counted.

Patient population. IC-containing sera from 10 patients with SLE were selected from sera tested in the Clinical Rheumatology Laboratory for IC levels by modified Raji cell radioimmunoassay. The serum from each patient containing the highest levels of ICs was selected. Sera were separated immediately after clotting was complete, stored at −90°C, and thawed only once immediately prior to analysis.

All patients were being followed for SLE in adult or paediatric clinics at the Billings and LaRabida Hospitals of the University of Chicago. Clinical records of all patients whose sera were tested were reviewed. Each patient selected met at least four of the preliminary criteria defined by the American Rheumatism Association (Cohen *et al.*, 1971). Additionally, sera from each patient at some time during the patient's course gave positive tests for anti-nuclear antibodies (at a dilution >1:20) and for double-stranded DNA antibodies by Farr radioimmunoassay employing chromatographically purified radiolabelled test DNA (Locker *et al.*, 1977). Seven of the 10 sera were hypocomplementaemic. Seven of the 10 patients had IgG at the dermal–epidermal junction as determined by indirect immunofluorescence of skin biopsy specimens and seven who underwent renal biopsies had changes consistent with lupus nephritis.

Immune complex determinations. IC levels in sera were determined by Raji cell radioimmunoassay (Theofilopoulos *et al.*, 1976) with modifications (Mariotti *et al.*, 1979).

Raji cells. Raji cells were cultured in RPMI 1640 minimal essential medium enriched with 10% fetal calf serum (GIBCO, Grand Island, New York) and containing antibiotics. Raji cell cultures were split at least twice a week, and immediately prior to use in the assay were counted in a haemocytometer and tested for viability by trypan blue exclusion.

Radioimmunoassay for immune complexes. Polypropylene Eppendorf tubes (1·5 ml) were precoated by incubation with RPMI+G at 20°C for 10 min. Assays were performed in duplicate; test-tube controls which contained all ingredients except Raji cells were included. Twenty-five microlitres of SLE serum were added to coated tubes employing 25-μl capillary micropipettes throughout (Clay-Adams, Parsippany, New Jersey). After mixing, 2×10^6 Raji cells in 50 μl of RPMI+G were added and the reaction mixture incubated at 37°C for 45 min. In the case of test-tube controls, 50 μl of RPMI-G was added instead of Raji cells. Following incubation, samples

and controls were washed three times with RPMI + G by repeated centrifugation and decantation. After the third decantation, 100 μl of a 10 μg/ml solution of ^{125}I-mRF (Mariotti *et al.*, 1979) in RPMI + G (approx. 1×10^6 counts per 5 min) was added to each tube. Samples and controls were washed three times with RPMI + G by centrifugation and decantation as before. After the third decantation, tubes containing Raji cell pellets were counted. Results were expressed as μg aggregated human globulin G (AHG) equivalent per ml serum by reference to a standard curve generated by assaying 25 μl of increasing concentrations of a standard preparation of AHG (mean sedimentation coefficient 50S) preincubated with 25 μl of fresh normal human serum as a complement source (Antel *et al.*, 1980).

Complement activities. Total haemolytic complement (CH50) was determined by standard methods (Kabat & Mayer, 1961). C4, C3c, and factor B levels were determined by radial immunodiffusion (Gewurz & Suyehira, 1976). CRA was determined using procedures which have been described previously (Miller & Nussenzweig, 1975).

RESULTS

The experimental design employed was as follows: endogenous ICs present in hypocomplementaemic SLE sera were allowed to bind to Raji cell receptors. After incubation and washing, the Raji cells, bearing ICs in their receptors, were then reincubated at 37°C with normal serum containing intact complement, or, alternatively, with zymosan-treated serum containing inactivated complement. The ability of complement to release the cell-bound ICs from the receptors was examined. In order to characterize the experimental system and to establish that, under the experimental conditions employed, CRA was demonstrable, studies with experimental ICs were first carried out. Experimental ICs were prepared *in vitro* from radiolabelled BSA and guinea-pig anti-BSA antiserum; the antiserum was heated to inactivate endogenous complement. Normal human serum from a healthy control was employed as a complement source.

Fig. 1 shows the results of experiments designed to determine the optimal antigen:antibody ratio and optimal complement concentration for maximal binding of the BSA:anti-BSA ICs to the Raji cells. ICs prepared in slight antibody excess (17 μg BSA) bound better than those prepared at other antigen:antibody ratios. Maximal binding (approx. 50%) was achieved with a 1:15 dilution of normal human serum as a complement source. Addition of lesser amounts or greater amounts of complement resulted in decreased binding. Addition of two and even four times the amount of ICs gave approximately the same per cent binding, demonstrating that the system was in receptor excess and that the per cent binding, therefore, was not influenced by the quantity of ICs added.

Fig. 2 shows the results of experiments designed to elucidate further the complement

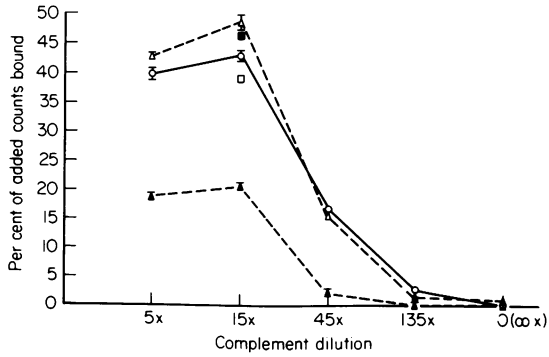

Fig. 1. Effect of antigen–antibody ratio and complement concentration on binding of BSA:anti-BSA complexes to Raji cells. Results are shown for complexes prepared in slight antibody excess, using 43 (▲- - -▲), 17 (△- - -△) and 7 μg BSA (o——o). The effect of adding two (■) and four (□) times the amount of immune complexes (prepared with 17 μg BSA) is also shown.

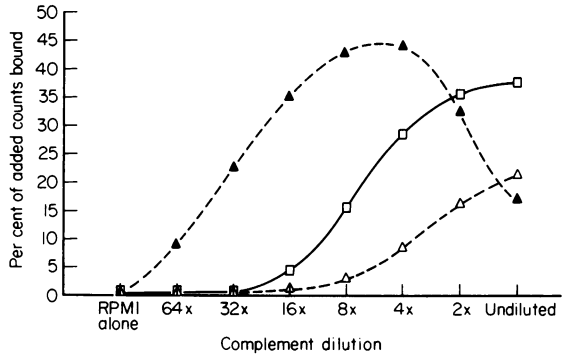

Fig. 2. Complement dependence of binding of BSA:anti-BSA complexes to Raji cells. Binding obtained using different dilutions of untreated (▲– – –▲), zymosan-treated (□——□) and heat-inactivated (56°C, 30 min) (△– – –△) normal human serum is shown.

dependence of the binding, and to establish that the effects observed were due to complement and were not influenced by the concentrations of other serum proteins. Complexes prepared with 17 μg BSA were used. Less binding to receptors was observed in the presence of insufficient or excess complement (untreated normal human serum). Eight to 16 times as much zymosan-inactivated serum and 32 times as much heat-inactivated serum was required to give equivalent binding to that obtained with fresh serum.

Experiments were carried out to determine the effect of incubation times on the binding results. Time of incubation of antigen with antibody was varied from 30 min to 12 hr. No difference was noted for times in excess of 30 min. Time of incubation of ICs (antigen:antibody) with optimal complement (1:15) was varied between 30 and 90 min. No significant difference was noted for times longer than 30 min. Finally, the time of incubation of ICs (antigen:antibody:complement) with Raji cells was varied between 45 and 135 min. Binding after 135 min was 14% greater than after 45 min. In all subsequent experiments, incubation times of 30, 30 and 45 min respectively were employed for the three above steps.

Fig. 3 shows the results of experiments designed to study the effect of complement on the BSA:anti-BSA ICs after they have bound to receptors, i.e. to demonstrate CRA. Standard ICs, prepared in slight antibody excess, were reacted with an optimal dilution of fresh normal human serum as a complement source and then with Raji cells. Washed IC-bearing Raji cells were then reincubated at 37°C with different dilutions of fresh serum or with zymosan-treated serum. Aliquots

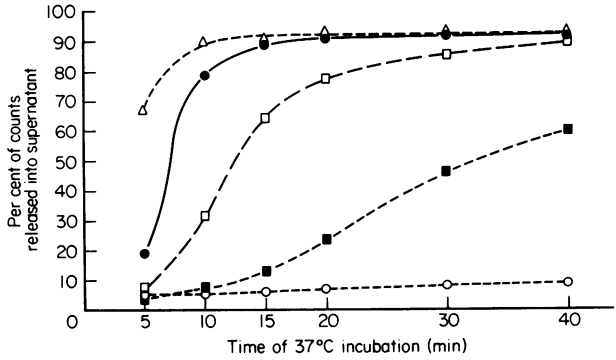

Fig. 3. Kinetics of BSA:anti-BSA complex release from Raji cells. Release after various times at 37°C in the presence of different dilutions of untreated or zymosan-treated normal human serum. (△– – –△) Undiluted untreated serum, (●——●) untreated serum, diluted ×2, (□– – –□) untreated serum, diluted ×4, (■– – –■) untreated serum, diluted ×8, (○– – –○) zymosan-treated serum.

of the reaction mixtures were removed at 0, 5, 10, 15, 20, 30 and 40 min and immediately cooled in ice to stop the reaction. In the presence of undiluted fresh serum, 90% of the receptor-bound ICs were released within 10 min; in the presence of zymosan-treated serum, on the other hand, less than 10% of the counts were released after 40 min. The greater the dilution of complement-containing serum, the greater the time required for release.

In experiments parallel to those above, ^{125}I-labelled AHG was used in place of the experimental ICs. One hundred micrograms of ^{125}I-AHG, after reaction with an optimal amount of complement, was incubated with 2×10^7 Raji cells. After washing, AHG-bearing Raji cells were reincubated with fresh and zymosan-treated serum as above. Contrary to results with BSA:anti-BSA complexes, there was no additional release of AHG by fresh serum over the background release observed for zymosan-treated serum.

Binding and release of experimental BSA:anti-BSA ICs was next studied employing unlabelled ICs. The ICs were subsequently quantitated using ^{125}I-labelled rabbit anti-guinea-pig Ig. These studies were done because similar conditions would have to be employed to study binding and release of endogenous ICs present in SLE sera.

Several experiments were performed to establish the optimal experimental conditions under which binding and release could be quantitated with radiolabelled antiglobulin. The optimal concentration of antiglobulin was determined. Proportionality between quantity of ICs added and counts of antiglobulin bound was established. A standard curve was generated for standard ICs prepared with an optimal dilution of fresh serum as complement source. Binding of antiglobulin was nearly linear over a 20-fold range of dilutions of standard ICs. Similar experiments were carried out with unlabelled AHG and endogenous SLE ICs using both rabbit ^{125}I-labelled anti-human globulin and, alternatively, purified ^{125}I-mRF. Proportionality was established for the human system for both antiglobulin reagents.

Fig. 4 shows that the kinetics of release of experimental ICs from receptors could be determined accurately employing unlabelled ICs followed by ^{125}I-anti-guinea pig Ig. There was good agreement between results obtained employing labelled antiglobulin and those determined employing labelled ICs. Identical experimental conditions, therefore, were employed in subsequent studies in which radiolabelled anti-human globulin was used to study endogenous ICs in SLE sera.

Ten sera from 10 different SLE patients with IC levels varying between 388 and 936 μg AHG Eq/ml by Raji cell assay (Mariotti *et al.*, 1979) were selected for release studies. Each IC-containing SLE serum was incubated with Raji cells and then washed in a fashion identical to that for experimental ICs. Raji cells, bearing SLE ICs in receptors, were then placed at 37°C in the presence of either fresh serum, or alternatively, zymosan-inactivated serum. Aliquots of the reaction mixture were removed after 0, 5, 10, 15, 25 and 40 min of incubation at 37°C and immediately cooled to 0°C. Percentage of ICs released from receptors after progressively increasing times was determined by quantitation of ICs remaining bound to Raji cells by addition of antiglobulin (^{125}I-mRF) after washing.

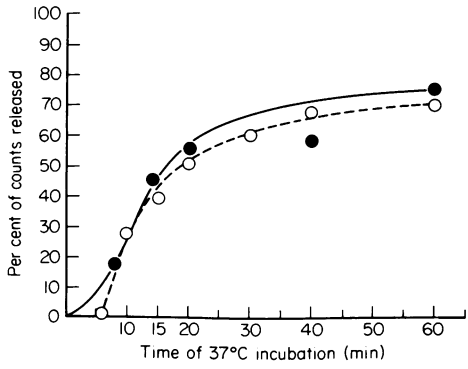

Fig. 4. Quantitation of release of unlabelled BSA:anti-BSA complexes using ^{125}I-labelled anti-guinea-pig Ig (●——●). Kinetics of release of ^{125}I-labelled BSA:anti-BSA complexes (o– – –o) are shown for comparison.

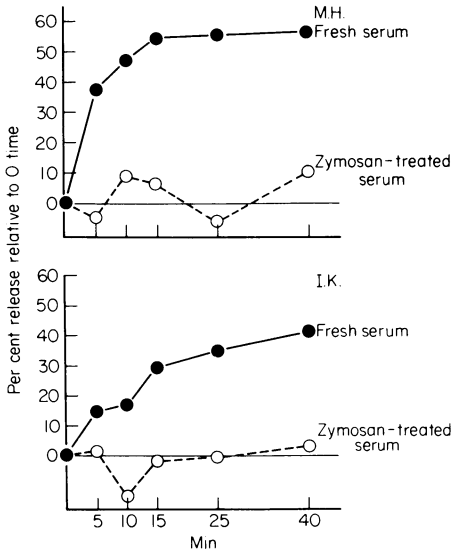

Fig. 5. Complement-dependent release from Raji cells of circulating immune complexes in sera from two SLE patients, M.H. and I.K., in an active phase. Background release in the presence of zymosan-treated serum is also shown.

The results obtained using IC-containing sera from two of the SLE patients are shown as examples in Fig. 5. In the case of M.H. 50% of the ICs were released by fresh serum in 15 min; only 15% were released by zymosan-treated serum after 40 min. In the case of I.K., > 40% of the ICs were released by fresh serum; although in this case release was slower, > 5% of the ICs were released by zymosan-treated serum in the same time period.

Similar results were obtained for ICs in sera from the other eight SLE patients. These results are summarized in Table 1. Release by fresh serum varied from a maximum of 58% to a minimum of 25%. In all cases, release by fresh serum was significantly higher than by zymosan-treated normal human serum. Release of unlabelled BSA:anti-BSA ICs quantitated using ^{125}I-antiglobulin in a

Table 1. Complement-dependent release of complexes in 10 SLE sera from Raji cell receptors

		Per cent release after 45 min at 37°C	
	Raji cell value (μg AHG Eq/ml)	Zymosan serum	Fresh serum
D.J.	480	25	58
M.H.	412	5	55
G.T.	766	18	52
L.H.	817	25	50
I.K.	780	2	40
E.M.	936	20	40
S.A.	480	22	40
S.K.	820	18	32
P.S.	388	0	25
D.G.	640	10	25
BSA:anti-BSA complexes*		20	65

* Quantitated using ^{125}I-anti-guinea-pig Ig.

parallel control study under identical conditions is shown for comparison. IC levels are given in the left-hand column for each serum. No simple correlation was found between the ability of ICs to release and IC level.

IC levels and ability to release were correlated with CH50s and antigenic C3, factor B and C4 levels for the cases in which complement measurements were performed immediately after venipuncture. No consistent relationships were found.

DISCUSSION

Our results using experimentally prepared BSA : anti-BSA ICs demonstrate that the binding of such ICs to receptors on human mononuclear cells is entirely complement-dependent. No binding was observed in the absence of added complement. Greatly decreased binding occurred if complement was inactivated by heating or zymosan pretreatment, indicating that the effect observed was not due to other serum proteins. Binding was most efficient over a restricted range of complement concentrations. At complement concentrations lower or higher than the optimum, reduced binding was observed. In preliminary studies, ICs were prepared at various antigen : antibody ratios; those prepared in slight antibody excess (approx. five-fold) bound best and were used for the remainder of the studies. When additional fresh complement was added to ICs in Raji cell receptors that were formed with optimal amounts of complement for binding, ICs were released from the receptors back into the fluid phase. Release occurred at 37°C, but not 4°C. The rate of release was a function of the amount of complement added. Insignificant release was observed in the presence of zymosan-treated serum.

These results are consistent with previous findings that interactions between ICs and cellular membrane receptors are modulated by complement (Miller & Nussenzweig, 1974; Oger & Medof, 1980). Whether ICs are bound to cellular receptors or are free in the soluble phase depends upon the quantity of complement that is present. At low levels of complement, ICs fix insufficient complement to permit binding to membrane receptors and therefore remain in the soluble phase. At higher levels of complement, ICs bind via fixed complement components to receptors. At yet higher levels of complement, or, alternatively, over extended periods of time in lesser amounts of complement, bound ICs are released from cellular membrane receptors and returned to the soluble phase. Thus, a dynamic state exists in which ICs pass through distinct stages, each having different biological properties.

Our results using unlabelled BSA : anti-BSA ICs prepared in an identical manner to the labelled ICs and using ^{125}I-rabbit anti-guinea-pig Ig antibody for their detection, demonstrated that complement-dependent release of unlabelled ICs could be quantitated accurately. Endogenous ICs present in sera of SLE patients in an active phase were allowed to bind to Raji cells under identical conditions. Release of these endogenous ICs was studied in parallel with release of the experimental unlabelled BSA : anti-BSA ICs, using ^{125}I-mRF and ^{125}I-anti-guinea-pig Ig respectively.

Release of endogenous ICs was demonstrable in all cases studied. Some release of the endogenous SLE ICs by zymosan-pretreated serum was observed in certain cases, but release by fresh serum was substantially greater in all cases. The kinetics of release were similar to those observed for the experimental ICs. Neither magnitude nor rate of release of ICs correlated well with CH50 or antigenic C3c, C4, or factor B levels of sera in which the ICs were present, or in four cases studied with CRA.

We conclude that intact complement can alter ICs that are present in hypocomplementaemic SLE sera and influence the relationship between these ICs and cellular membrane receptors. Specifically, intact complement can release pathological ICs present in SLE sera from receptors on human mononuclear cells. Previous studies (Miller & Nussenzweig, 1974; Takahashi *et al.*, 1976) suggest that these ICs are permanently altered and cannot rebind.

Complement-dependent release has been studied previously using several different experimental antigen : antibody systems (Miller *et al.*, 1973; Czop & Nussenzweig, 1976; Oger & Medof, 1980). The present work strongly suggests that the same phenomenon operates for naturally-occurring ICs. Previous studies indicate that this phenomenon is not a consequence of competition for

receptors by C3 split products. Addition of C3b to genetically C3-deficient serum does not induce release, whereas addition of native C3 to C3-deficient serum does (Miller *et al.*, 1973). The release phenomenon can be equally well demonstrated in a solid-phase system which contains no cellular C3 receptors whatsoever (Miller, 1977). By analogy to previous studies using experimental ICs (Miller & Nussenzweig, 1975; Czop & Nussenzweig, 1976), the endogenous SLE complexes may be converted during release into partially dissociated aggregates which are smaller than the original ICs.

Our results have a number of clinical implications. If ICs exist as a series of different forms having different biological properties, these different forms may not have the same pathological significance. Little is known about the state of circulating ICs in SLE with regard to where they exist along the sequence of reactions with complement. Clearly, identification of which forms are pathogenic would be of obvious clinical value. It is possible that different forms may either collect at different sites or may induce different types of inflammation. For example, complement receptors have been detected on glomerular cells in the kidney (Shin *et al.*, 1977).

Our results raise the possibility that hypocomplementaemia secondarily due to consumption of complement by ICs may contribute to the persistence of the ICs. If the hypocomplementaemic state creates a condition in which ICs are hindered from further progress along the normal complement reaction sequence which would normally result in their elimination, a vicious cycle may be created. Persistence of antigen and ongoing production of new ICs would tend to maintain the hypocomplementaemic state and perpetuate this cycle.

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REFERENCES

ANTEL, J.P., MEDOF, M.E., OGER, J.J.-F., KUO, H.H. & ARNASON, B.G.W. (1980) Induction of human suppressor cell activity by heat-aggregated human immunoglobulin-G (AHG). *Clin. Res.* **28,** 39a.

AGNELLO, V., WINCHESTER, R.J. & KUNKEL, H.G. (1970) Precipitin reactions of the C1q component of complement with aggregated γ-globulin and immune complexes in gel diffusion. *Immunology*, **19,** 909.

COHEN, A.S., REYNOLDS, W.E., FRANKLIN, P.J., KULKA, M.W., ROPES, L.E., SHULMAN, L.E. & WALLACE, S.E. (1971) Preliminary criteria for the classification of systemic lupus erythematosus. *Bull. rheum. Dis.* **21,** 643.

CZOP, J. & NUSSENZWEIG, V. (1976) Studies on the mechanism of solubilization of immune aggregates by complement. Assembly of a Factor B-dependent C3-convertase on the immune complexes. *J. exp. Med.* **145,** 86.

EDEN, A., BIANCO, C. & NUSSENZWEIG, V. (1973) Mechanism of binding on soluble immune complexes to lymphocytes. *Cell. Immunol.* **7,** 459.

GEWURZ, H. & SUYEHIRA, L.A. (1976) Complement. In *Manual of Clinical Immunology* (ed. by N. R. Rose and H. Friedman), pp. 37–38. American Society for Microbiology, Washington, DC.

KABAT, E.A. & MAYER, M.M. (1961) *Experimental Immunochemistry*, p. 135. Charles C. Thomas, Springfield, Illinois.

LOCKER, J.D., MEDOF, M.E., BENNETT, R.M. & SUKHUPUNYARAKSA, S. (1977) Characterization of DNA used to assay sera for anti-DNA antibodies; determination of the specificities of anti-DNA antibodies in SLE and non-SLE rheumatic disease states. *J. Immunol.* **118,** 694.

MCCONAHEY, P.J. & DIXON, F.J. (1966) A method of trace iodination of proteins for immunologic studies. *Int. Arch. Allergy appl. Immunol.* **29,** 185.

MARIOTTI, S., DEGROOT, L.J., SCARBOROUGH, D. & MEDOF, M.E. (1979) Study of circulating immune complexes in thyroid disease. Comparison of Raji cell RIA and specific thyroglobulin–anti-thyroglobulin radioassay. *J. clin. Endocrinol. Metab.* **49,** 679.

MAURER, P.H. (1971) The quantitative precipitin reaction. In *Methods in Immunology and Immunochemistry* (ed. by C. R. Williams and M. W. Chase), p. 1. Academic Press, New York and London.

MILLER, G.W. (1977) Complement-mediated dissociation of antibody from immobilized antigen. *J. Immunol.* **119,** 488.

MILLER, G.W. & NUSSENZWEIG, V. (1974) Complement as a regulator of interactions between immune complexes and cell membranes. *J. Immunol.* **113,** 464.

MILLER, G.W. & NUSSENZWEIG, V. (1975) A new complement function: solubilization of antigen–antibody aggregates. *Proc. Natl. Acad. Sci. USA*, **72,** 418.

MILLER, G.W., SALUK, P.H. & NUSSENZWEIG, V. (1973) Complement-dependent release of immune complexes from the lymphocyte membrane. *J. exp. Med.* **138,** 495.

NYDEGGER, U.E., LAMBERT, P.H., GERBER, H. & MIESCHER, P.A. (1974) Circulating immune complexes in the serum in systemic lupus erythematosus and in carriers of hepatitis B antigen. Quantitation by binding to radiolabeled C1q. *J. clin. Invest.* **54,** 297.

OGER, J.J.-F. & MEDOF, M.E. (1980) A role for human

red blood cells in handling of immune complexes in man. *Clin. Res.* **28,** 507a.

SHIN, M.L., GELFAND, M.C., NAGLE, R.B., CARLO, J.R., GREEN, I. & FRANK, M.M. (1977) Localization of receptors for activated complement on visceral epithelial cells of the human renal glomerulus. *J. Immunol.* **118,** 869.

TAKAHASHI, M., CZOP, J., FERREIRA, A. & NUSSENZWEIG, V. (1976) Mechanism of solubilization of immune aggregates by complement. Implications for immunopathology. *Transplant. Rev.* **32,** 121.

TAKAHASHI, M., TACK, B.F. & NUSSENZWEIG, V. (1977) Requirements for the solubilization of immune aggregates by complement. Assembly of a Factor B-dependent C3-convertase on the immune complexes. *J. exp. Med.* **145,** 86.

TAKAHASHI, M., TAKAHASHI, S., BRADE, V. & NUSSENZWEIG, V. (1978) Requirements for the solubilization of immune aggregates by complement. The role of the classical pathway. *J. clin. Invest.* **62,** 349.

THEOFILOPOULOS, A.N., DIXON, F.J. & BOKISCH, V.A. (1974) Binding of soluble immune complexes to human lymphoblastoid cells. I. Characterization of receptors for IgG, Fc and complement and description of the binding mechanism. *J. exp. Med.* **140,** 877.

THEOFILOPOULOS, A.N., WILSON, C.B., BOKISCH, V.A. & DIXON, F.J. (1974) Binding of soluble immune complexes to human lymphoblastoid cells. II. Use of Raji cells to detect circulating immune complexes in animal and human sera. *J. exp. Med.* **140,** 1230.

THEOFILOPOULOS, A.N., WILSON, C.B. & DIXON, F.J. (1976) The Raji cell radioimmune assay for detecting immune complexes in human sera. *J. clin. Invest.* **57,** 169.