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Detection and partial characterization of immune complexes in patients with rheumatoid arthritis plus Sjogren's syndrome and with Sjogren's syndrome alone

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SUMMARY

In order to characterize the immune complexes detected in patients with Sjogren's syndrome (SS) and with rheumatoid arthritis (RA), the sera of 19 patients with SS alone and 11 with SS plus RA were examined. Elevated quantities of circulating immune complexes (CIC) were detected in 67% by the C1q-binding assay (C1q-BA), 73% by the C1q-solid phase (C1q-SP) assay, 43% by the monoclonal rheumatoid factor solid phase assay (mRF-SP) and 33% by the mRF-inhibition assay (MRF-Inh). Elevated concentrations of IgM RF were detected in 83% and of IgG RF in 73% of the sera by radioimmunoassay. Strong correlations existed between RF of the IgM and IgG classes and both the C1q-BA and the C1q-SP. Three lines of evidence indicated that RF were important components of the immune complexes detected by these radioimmunoassays. These results indicated that in those patients with RA plus SS, as well as those with SS alone, both IgM and IgG RF made substantial contributions to immune complexes detected both by the C1q-BA and the C1q-SP.

INTRODUCTION

The use of sophisticated radioimmunoassays has recently resulted in the detection of abundant quantities of circulating immune complexes in the majority of patients with Sjogren's syndrome (SS) alone as well as those with rheumatoid arthritis (RA) associated with SS (Lawley *et al.*, 1979; Fischbach *et al.*, 1980; Berne & Lawless, 1979). The importance of immune complexes in the pathogenesis of these disorders is not clear. Fischbach *et al.* (1980) failed to observe any relationship between the degree of activity of SS as measured by lymphocytic infiltration of labial gland biopsies and immune complexes as measured by the Raji cell assay. However, immune complex manifestations are common in patients with SS including cryoglobulinemia (Zinneman & Caperton, 1977; Aizawa *et al.*, 1979), hyperviscosity syndrome (Blaylock, Waller & Normansell, 1974; Alarcon-Segovia *et al.*, 1974), immune complex mediated renal disease (Moutsopoulos *et al.*, 1978; Winer *et al.*, 1977), and purpuric vasculitis (Aizawa *et al.*, 1979; Moutsopoulos *et al.*, 1979a & b). Immune complexes may also mediate effects in patients with SS in other ways. Evidence suggests that immune complexes may in part be responsible for defects in reticuloendothelial clearance (Moutsopoulos *et al.*, 1980) and for defects noted in suppressor T cell ($T\gamma$) function in patients with

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SS (Moutsopoulos & Fauci, 1980). Additionally in those patients with RA plus SS, like those with RA alone, the articular as well as other extraarticular manifestations, may be due to immune complexes (Nydegger *et al.*, 1977; Zvaifler, 1973; Abel *et al.*, 1980).

In order to better characterize the immune complexes found in these patients, four commonly employed radioimmunoassays were employed to detect immune complexes. In addition, sensitive class-specific radioimmunoassays were used to detect IgM and IgG rheumatoid factor (RF). Since both IgG and IgM RF significantly correlated with immune complexes detected employing C1q, the contribution of the RF to the detected immune complexes was examined in detail.

MATERIALS AND METHODS

Patient sera. The sera of 30 patients with SS were examined, 17 from Mexico D.F. and 13 from San Antonio, Texas. Nineteen patients had SS alone diagnosed by clinical symptoms of the sicca complex, particularly dry eyes and dry mouth, ophthalmologic examination including Shirmer's test, rose bengal staining and slit lamp examination and/or labial gland biopsy. Eleven patients had SS plus definite or classical RA. Two of these patients had developed vasculitis at some time during their course. Patients with SS alone and with SS plus RA fulfilled established criteria for the diagnosis of SS (Block *et al.*, 1965). Normal control sera were obtained from university employees. Blood was drawn, clotted at room temperature and stored at $-20°C$ until use.

Immune complex assays. The performance of the immune complex assays by us has been described in detail (Yoshinoya, Cox & Pope, 1980; Yoshinoya & Pope, 1980). Briefly the C1q-binding assay (C1q-BA) employed polyethylene glycol and was designed after that described by Zubler *et al.* (1976). The C1q-solid phase (C1q-SP) and the monoclonal rheumatoid factor solid phase (mRF-SP) assays were designed after the method of Hay, Nineham & Roitt (1976). Briefly, polystyrene tubes were coated with C1q or mRF. Sera were added and bound complexes detected with a ^{125}I-F(ab')$_2$ rabbit anti-human Fab(γ). The mRF-inhibition assay (mRF-Inh) was performed by coupling human IgG to para-azobenzamidoethyl Sepharose 4B. A mRF was radiolabelled and the ability of test serum to inhibit its binding to the solid phase was calculated as a percent of the total bound (Yoshinoya & Pope, 1980). When the results of the assays were compared, they were reported as an immune complex index (Yoshinoya *et al.*, 1980; Yoshinoya & Pope, 1980), obtained by dividing each patient value by the normal mean for that assay.

The C1q-SP assay was modified to detect immune complexes possessing RF activity (C1q-SP-RF). The assay was performed as already described except that ^{125}I-normal pooled human IgG (Cohn Fraction II) was used in place of anti-Fab(γ). RF containing immune complexes were quantitated as ng of ^{125}I-IgG bound.

Rheumatoid factor assays. These assays were performed by radioimmunoassays as previously described by us (Pope & McDuffy, 1979; 1981). Rabbit IgG was employed as the antigen to detect IgG-RF, while human IgG was used to detect IgM-RF. Quantitation of IgG-RF was performed with ^{125}I-anti-Fab(γ) and IgM RF with ^{125}I-anti-human μ.

Absorption of rheumatoid factor. In an attempt to remove both IgM- and IgG-RF, test sera were incubated with a Sepharose 4B-human IgG immunoadsorbant. Sera adsorbed with Sepharose 4B conjugated with human serum albumin (HSA) served as the control for each sample. Two-tenths millilitre of serum and 2·0 ml of a 50% suspension of IgG-Sepharose or HSA-Sepharose were tumbled together for 48 hr at 4°C. The immunoadsorption was carried out in PBS-BSA plus 1 mM EDTA. Samples were centrifuged at 1500 ***g*** for 5 min. The supernatants were harvested and the immunoadsorbant washed once with 1·8 ml of PBS-BSA. This wash was added to the original and these were employed to perform the assays for RF and immune complexes.

Recombination experiments. Sera (1·2 ml) were applied to a Sepharose 6B column (2·6 cm × 90 cm) in a 0·05 M sodium acetate, 0·15 M sodium chloride, pH 3·5 buffer. The 19S and 6·6S fractions were pooled and dialysed against Tris buffer, pH 8·0. The 6·6S peak was used without further manipulation in order to avoid the artificial production of immune complexes by aggregation of IgG. The 19S peak was concentrated to a volume of 2–3 ml. To increasing volumes of the 6·6S peak

(ranging from 0·0 to 0·534 ml) 0·21 ml of the concentrated 19S peak was added. The volume in each tube was brought up to 1 ml and 0·45 ml was employed for the C1q-SP.

RESULTS

Immune complex assays

The C1q-BA was positive in 67% of the sera, the C1q-SP in 75%, the mRF-SP in 43% and the mRF-Inh in 33%. The distribution of values of all the patients for each assay is graphically represented in Fig. 1. Values were broadly distributed for patients with RA plus SS as well as with

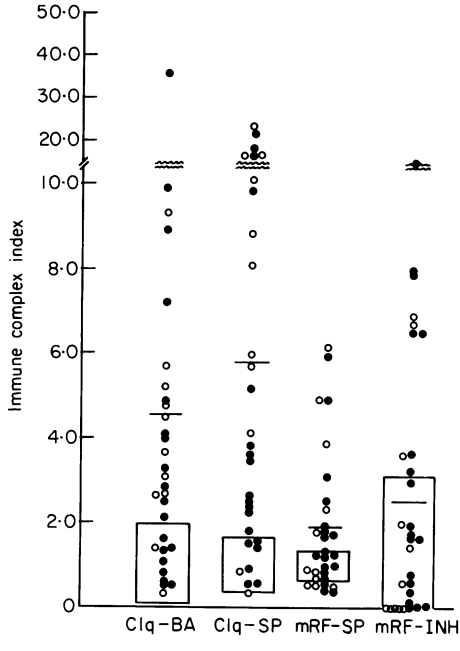

Fig. 1. Detection of immune complexes in patients with SS alone (●) and SS plus RA (○) by four radioimmunoassays. The results are recorded as an immune complex index obtained by dividing each patient value by the normal mean for that assay. Therefore, the normal mean for each assay is 1. The rectangles define the normal mean ± 2 s.d. for each assay. The horizontal lines represent the patient means for each assay.

SS alone, and there were no statistical differences either in the frequency or in the degree of elevation between either group. The patient values for both assays employing C1q were significantly different from the normal controls (Table 1). Six sera were abnormal by all four assays, nine by three assays, four by two and six by one. Five sera were normal by all four assays. Correlations, corrected (Pc) for the number of potentially dependent variables (Pope & Yoshinoya, 1981), were noted between several of the assays. The C1q-BA correlated with the C1q-SP ($r = 0{\cdot}69$, $Pc < 0{\cdot}009$) and with the mRF-Inh ($r = 0{\cdot}58$, $Pc < 0{\cdot}009$) while the C1q-SP correlated with the mRF-SP ($r = 0{\cdot}73$, $Pc < 0{\cdot}009$).

Rheumatoid factor assays

Eighty-three percent of the sera were positive for IgM RF and 73% were positive for IgG RF. While IgM RF was not significantly different between the groups, the IgG RF was significantly greater in the group with RA plus SS ($P < 0{\cdot}04$). The IgM and IgG RF of both groups, as well as the combined group, were significantly greater than the normal controls ($P < 0{\cdot}0009$). Five of the sera were

Table 1. Patient and control values for immune complexes and rheumatoid factor

		Immune complex assays				Rheumatoid factor assays	
	*n**	C1q-BA (% bound)	C1q-SP (ng Anti-Fab [γ])	mRF-SP (ng Anti-Fab [γ])	mRF-Inh (% Inh)	IgM-RF (ng Anti-μ)	IgG-RF (ng Anti-Fab [γ])
Control	15	1·88±0·23†	4·84±0·40	12·6±0·57	4·09±1·10	0·72±0·12	0·35±0·04
SS Total	30	8·58±2·25	28·0±5·25	23·7±3·83	10·3±2·62	10·6±1·79	4·13±1·73
		(67%)‡	(73%)	(43%)	(33%)	(83%)	(73%)
		(0·001)§	(0·0009)	(n.s.)	(n.s.)	(0·0009)	(0·0009)
RA+SS	19	9·26+3·49	22·0+6·25	22·2+4·25	12·6+3·46	9·77±2·41	4·76±2·73
		(58%)	(68%)	(42%)	(37%)	(79%)	(63%)
		(0·001)	(0·001)	(n.s.)	(n.s.)	(0·0009)	(0·0009)
SS Alone	1	7·40±1·40	38·3±8·93	26·4±7·67	6·36±3·83	12·1±2·64	3·05±6·64
		(82%)	(82%)	(45%)	(27%)	(91%)	(91%)
		(0·005)	(0·0009)	(n.s.)	(n.s.)	(0·0009)	(0·0009)

* Number of sera examined.
† Mean ± s.e.m.
‡ Percentage of sera > 2 s.d. above the normal mean.
§ Significance of difference from the normal controls by the Mann-Whitney test.

negative for both IgM RF and IgG RF while three additional sera were positive for IgM RF but negative for IgG RF. We found no patients who were positive for IgG RF but negative for IgM RF.

Relationship of immune complexes, rheumatoid factors and immunoglobulin concentration
The relationship between immune complexes and RF was examined. IgM RF correlated with the C1q-BA ($r=0·53$, $Pc<0·009$), the C1q-SP ($r=0·49$. $Pc<0·03$) (Fig. 2) and the mRF-Inh ($r=0·58$, $Pc<0·009$) assays. The IgG RF correlated with the C1q-BA ($r=0·58$, $Pc<0·009$), and the C1q-SP ($r=0·68$, $Pc<0·009$) (Fig. 2). As mentioned earlier, five of the patients had no immune complexes

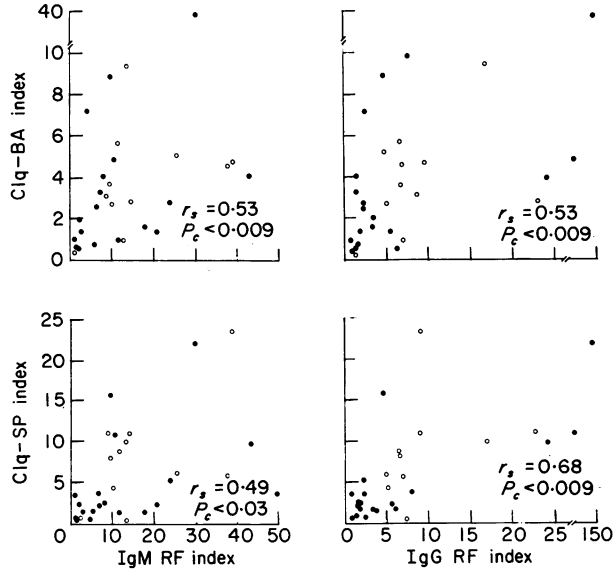

Fig. 2. Relationship of immune complexes detected by the C1q-BA and the C1q-SP and RF of the IgM and IgG classes. Correlation coefficients (r_s) were determined by the Spearman's rank test. The RF indexes were derived by dividing each patient value by the normal mean for that assay. (● = SS alone; ○ = SS + RA).

detected by any assay and five were negative for both IgM and IgG RF. Three sera were negative by all six assays. Two sera were negative for both IgM and IgG RF; one of these was weakly positive for immune complexes by one assay while the other was positive by two assays. Two sera were positive for both IgM and IgG RF but were negative for immune complexes by all four assays. These data clearly indicated that RF were not entirely responsible for the immune complexes detected. Nevertheless, there appeared to be a strong association between RF and immune complexes detected by several of the assays, particularly those utilizing C1q.

The relationship of IgG and IgM concentration to all six radioimmunoassays was also examined. IgG concentration did not correlate with any assay except weakly with the mRF-SP ($r=0{\cdot}39$, $P<0{\cdot}02$) and the mRF-Inh ($r=0{\cdot}40$, $P<0{\cdot}02$). IgM concentration correlated only with the C1q-BA ($r=0{\cdot}59$, $P<0{\cdot}002$).

Partial characterization of immune complexes

In order to examine the contribution of RF to the detected immune complexes, a Sepharose 4B-human IgG immunoadsorbant was employed with a Sepharose 4B-HSA as the control. The results of the adsorption of the individual sera are represented in Fig. 3. The IgM RF decreased a mean of 83%, the IgG RF 23% and the C1q-BA 51%. As noted in Fig. 3, the changes of the C1q-BA and the IgM RF were very comparable for four of the five sera. The discrepancy noted for the other patient (SS patient, fourth from the left, Fig. 3) was likely due to the extraordinarily increased concentration of IgG RF which was little affected by the adsorption with human IgG. Excluding this patient, the C1q-BA decreased 75% and the IgM RF 88%. The very slight changes noted with the C1q-SP assay following adsorption with 4B-IgG were due in part to the fact that the adsorption with the 4B-HSA reduced the values for the C1q-SP a mean of 69% compared to dilution alone. No consistent change in RF activity or in the C1q-BA was noted by adsorption with Sepharose 4B-HSA. In other studies not recorded here, Sepharose alone as well as Sepharose coupled with BSA or glycine gave results similar to Sepharose-HSA.

Therefore, an alternative method was utilized to study the effects of the removal of RF on immune complex detection. Sera were adsorbed with glutaraldehyde aggregated IgG (Avrameas & Ternynck, 1969) and the results compared with sera handled in buffer alone. As in the experiments employing Sepharose-IgG, in four of the five patients' sera, the fall of IgM RF was associated with a similar reduction of the C1q-BA (57% and 56% respectively for the four sera). Immune complexes

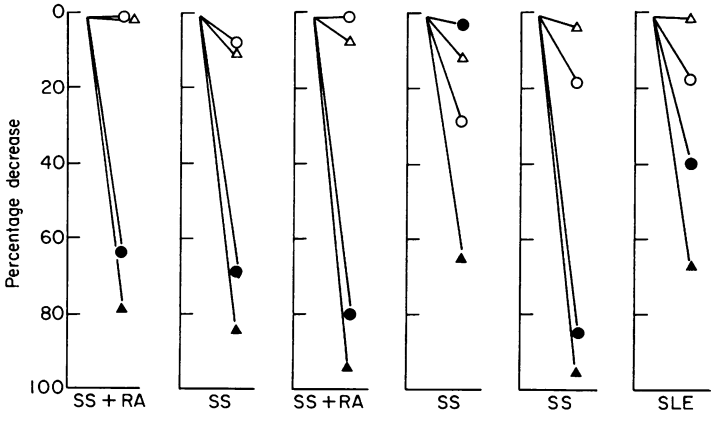

Fig. 3. Effect of adsorption of RF by a Sepharose 4B-human IgG immunoadsorbant on the detection of immune complexes. Five sera from patients with SS alone or SS plus RA and one from a patient with systemic lupus erythematosus, negative for RF by the latex agglutination test, were examined. Each assay is represented by a different symbol: C1q-BA (●), C1q-SP (○), IgM-RF (▲) and IgG-RF (△). A line drawn through the symbol indicates that a slight increase was noted following absorption. The initial or baseline value for each assay was determined following adsorption with Sepharose 4B-HSA. The solid line connecting each figure with 0%, represents the change noted following adsorption with Sepharose 4B-IgG compared to 4B-HSA.

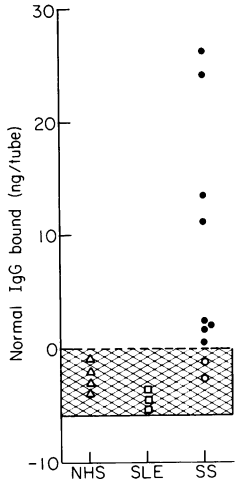

Fig. 4. C1q-SP-RF assay results. The assay was performed as described in the Materials and Methods. The results are recorded as the ng/tube of ^{125}I-normal human IgG bound. The open symbols represent the sera of patients or normal controls which were negative for RF by the latex agglutination test. The hatched area represents the mean $\pm$ 2 s.d. for all the RF negative sera.

detected by the C1q-SP recorded a mean decline of 35% following adsorption with aggregated IgG from 26·9 $\pm$ 9·77 (s.e.m.) ng anti-Fab(γ)/tube to 17·5 $\pm$ 5·51 ng/tube.

The adsorptive studies indicated that removal of IgM RF was associated with comparable changes for the C1q-BA. The effects of RF on the C1q-SP assay were less clear. Therefore, the C1q-SP assay was modified to directly detect RF involved in the formation of immune complexes (Fig. 4). The assay (C1q-SP-RF) was based on the observation that RF bound to the C1q as an immune complex or as a component of an immune complex was still capable of binding free IgG. The assay was performed in the usual fashion with the exception that ^{125}I-human IgG was used in place of the F(ab')$_2$ rabbit antiserum. As can be seen, all eight RF positive sera from Sjogren's patients gave values that were greater than the mean plus 2 s.d. for nine RF negative sera, including two from RF negative patients with SS and four from RF negative patients with SLE.

In order to further document the significance of RF on the immune complex assays under study, patient and control sera were eluted over a Sepharose 6B column at pH 3·5. The 6·6S and the 19S peaks were pooled and dialysed. For the patient with SS, the addition of the 19S peak to the 6·6S

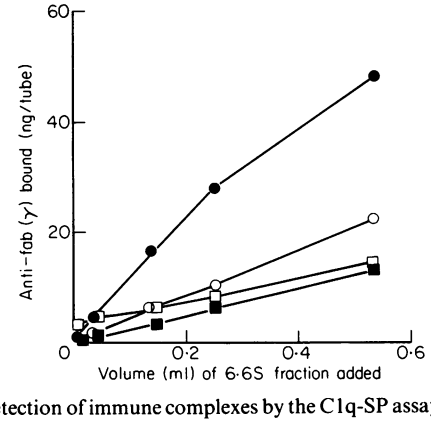

Fig. 5. Augmentation of the detection of immune complexes by the C1q-SP assay following reassociation of 6·6S and 19S peaks. See text and Materials and Methods for details. All values recorded for combination of the 19S and 6·6S peaks was obtained by first subtracting the value for the 19S peak alone. (●——● = SS-6·6S + 19S; O——O = SS-6·6S alone; ■——■ = NHS-6·6S + 19S; □——□ = NHS-6·6S alone).

peak resulted in augmentation of immune complexes detected by the C1q-SP (Fig. 5). No such augmentation was noted using the 19S and the 6·6S fractions obtained following gel filtration of normal human serum. Such effects were not noted with every RF positive serum. We examined another SS serum in a similar fashion and found no augmentation while the serum of a patient with RA alone showed comparable augmentation.

DISCUSSION

This study confirms the high incidence of circulating immune complexes both in patients with SS alone and in those with classical or definite RA associated with SS. Those assays which were dependent on complement (C1q) or complement receptors (Raji cells) were more sensitive than those using mRF (Lawley *et al.*, 1979; Fischbach *et al.*, 1980; Berne & Lawless, 1979). The present study examined in detail the relationship between immune complex-like materials and the IgG and IgM classes of RF. A number of previous studies have noted an association between the presence of RF and the detection of complexes by the C1q-BA (Miller, Osborne & Hsu, 1980; Gupta *et al.*, 1979; Solling, Solling & Lassen, 1980; Holborow, Thompson & Howles, 1979). Further, several studies have observed correlations between the concentration of RF and the concentration of immune complexes detected by the C1q-BA (Lawley *et al.*, 1979; Berne & Lawless, 1979; Roberts-Thomson *et al.*, 1980; Clague & Holt, 1978; Erhardt, Mumford & Maini, 1979a & b). We noted significant associations between the C1q-BA and both IgM and IgG RF. No prior study has reported this relationship employing an assay specific for the IgM class of RF. One prior study (Claque & Holt, 1978) reported a correlation between the C1q-BA and IgG RF in RA while another noted no association in SS (Lawley *et al.*, 1979). Both studies which detected a relationship between the C1q-BA and IgG RF, employed rabbit IgG to detect IgG RF while Lawley *et al.* (1979) used human Fc. Recently, we demonstrated that rabbit IgG was more sensitive than human Fc to detect IgG RF by our radioimmunoassay (Pope & McDuffy, 1981), which may partially account for this difference.

The present study also demonstrated significant correlations between immune complexes detected by the C1q-SP and both IgM and IgG RF. One previous study noted a correlation between IgG RF and immune complexes detected by the C1q-SP in the sera and synovial fluids of patients with RA (Hay *et al.*, 1979). In a prior study we observed strong correlations ($r = 0{\cdot}58$–$0{\cdot}87$) between immune complexes detected by both the C1q-BA and C1q-SP and both IgM and IgG RF in the sera of 31 patients with RA (Pope *et al.*, 1981).

The relationship between the concentration of C1q binding immune complexes and RF suggested the possibility that RF may contribute to the formation of these complexes. Adsorption of RF, particularly IgM RF, was clearly associated with a comparable decrease of immune complexes detected by the C1q-BA. This observation was noted regardless of the technique employed to absorb the RF and implicates RF as a major component of the immune complexes detected by this assay. Although controversial (Lawley *et al.*, 1979), this conclusion is supported by the observation of Erhardt *et al.* (1979b) that both RF and complexes detected by the C1q-BA could be eluted from solid phase IgG which had been previously incubated in the sera of patients with RA. The relationship of IgG RF to the immune complexes detected by the C1q-BA was less clear since no immunoadsorbant was particularly efficatious in absorbing IgG RF with decreases ranging from 15 to 23%.

Interpretation of the adsorptive studies examining the effect of RF on the C1q-SP was complicated by the effects of the Sepharose itself. McDuffie, Hunder & Clark (1978) also noted that the adsorption of RA sera with Sepharose alone was associated with a decrease in complement fixing immune complex-like material in some cases. Whether or not the Sepharose was adsorbing immune complexes detected only by the C1q-SP assay, or was interfering with their detection in some other fashion remains to be determined. The contribution of RF to complexes detected by the C1q-SP assay was documented by three lines of investigation: adsorption of C1q-SP detectable complexes by glutaraldehyde aggregated IgG; the direct demonstration of RF activity in the complexes detected by the C1q-SP-RF assay; and finally, the augmented detection of complexes by

the C1q-SP by combination of 6·6S and 19S RF containing fractions. Concerning the C1q-SP-RF assay, it was clear that only RF containing complexes were detected since lupus sera, strongly positive by the C1q-SP but negative for RF, were negative by the C1q-SP-RF assay. Therefore, we conclude that the RF positive SS sera studied contained C1q-SP detectable immune complexes possessing RF activity. A possible criticism of this interpretation might be that RF could bind to any immune complex already bound to the C1q-SP. If this were true, the RF would then be a significant component of that immune complex. The fact that the RF remained adherent even under the relatively dissociating conditions of the washing procedure indicated that the RF are indeed integral components of the immune complexes. This interpretation was further supported by comparing the results of the C1q-SP-RF with those noted following the absorption of RF by glutaraldehyde aggregated IgG. Pre- and post-absorptive studies had been performed on four of the eight RF positive samples used in C1q-SF-RF assay. The serum that gave the lowest C1q-SP-RF immune complex value, indicating only minimal involvement of RF in the C1q-SP detectable complexes, showed no decrease by the standard C1q-SP assay following absorption with glutaraldehyde aggregated IgG. On the other hand, the samples with C1q-SP-RF immune complex values of 2·14, 13·2 and 26·1 ng/tube noted reductions in the C1q-SP assay following adsorption with aggregated IgG of 27%, 53% and 56%, respectively.

The presence of RF in both peaks employed in the recombination experiments suggests the importance of both IgM and IgG RF in the immune complexes detected by the C1q-SP. Nevertheless, the possibility of recombination of other antigen–antibody systems during these reassociation experiments cannot be excluded. Further support for the importance of the interaction of IgM RF with IgG RF may be derived from the study by Jones *et al.* (1980), which suggested that IgM RF may in fact preferentially interact with IgG RF compared to normal IgG. These data strongly implicate RF, possibly both IgM and IgG, in the composition of the immune complexes detected not only by the C1q-BA but also by the C1q-SP assay in the sera not only of patients with SS alone but also in those with SS plus RA.

In patients with RA an understanding of composition of the immune complexes will be most helpful in clarifying the underlying pathophysiologic process since abundant evidence suggests that immune complexes may be responsible for much of the joint destruction, as well as the extra-articular manifestations (Nydegger *et al.*, 1977; Zvarfler, 1973; Abel *et al.*, 1980). The fact that the complexes described in this and many other studies were readily detected by assays employing C1q suggests that they have important phlogistic potential. In patients with SS alone, Fischbach *et al.* (1980) noted no correlation between circulating immune complexes detected by the Raji cell assay and the extent of the SS as determined by salivary gland focus scores. Nonetheless, certain manifestations of SS such as vasculitis, hyperviscosity syndrome and glomerulonephritis are likely due to immune complexes. Actually, patients with Sicca syndrome were noted in one study to have a greater instance of extraarticular manifestations thought due to circulating immune complexes such as Raynaud's phenomenon, purpura, myositis and renal involvement compared to those with RA alone (Moutsopoulos *et al.*, 1979a & b). Therefore, while immune complexes may not be responsible for the salivary gland inflammation noted in SS, they appear responsible for many of the systemic manifestations of this disorder as well as the articular and the extraarticular manifestations of patients with RA. Since RF make a significant contribution to the immune complexes detected, they may play a pivotal role in the pathogenesis of certain manifestations of these disorders.

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