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Molecular analysis of complement-fixing rheumatoid synovial fluid immune complexes

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SUMMARY

The characteristics of the solid-phase conglutinin method for the isolation of C3-containing complexes from the synovial effusions of rheumatoid arthritis patients were assessed. All major proteins in such complexes were identified and shown to be either immunoglobulin or complement components. The high proportion of IgM and the association between complexed IgM and latex agglutination titre suggest that IgM rheumatoid factor, probably binding to self-associated IgG antiglobulins, is of major importance in the formation of complement-fixing complexes. A minority of samples contained unidentified trace components and these differed from one fluid to another. The levels of complexed immunoglobulins were closely correlated to the titres of synovial fluid antiglobulins. The data accords with the view that autosensitization to IgG plays the primary role in the development of immunopathological features of established rheumatoid arthritis.

INTRODUCTION

In a previous report (Male, Roitt & Hay, 1980) we described the isolation of immune complexes from rheumatoid synovial fluids by centrifugation through a combined sucrose/polyethylene glycol gradient. The isolated complexes were analysed by a variety of techniques based on SDS-PAGE (Male & Roitt, 1979). The results implied that complex formation in these effusions could be largely accounted for by antiglobulins with the possibility of a small contribution from DNA/anti-ssDNA complexes in a minority of patients.

The various isolation methods only allow recovery of a proportion of the total spectrum of complexes occurring in any one patient or single disease, and the nature of the complexes recovered depends on the characteristics which are selected for by the particular technique employed. Although sucrose/PEG centrifugation leads to recovery of the majority of complexes larger than 15S, it appeared desirable to supplement the original data by an analysis of complexes isolated by an independent method. There is evidence that synovial fluid complexes are active in complement fixation (Winchester, Agnello & Kunkel, 1970) and that this then permits the complexes to interact with, and stimulate lymphocytes (Kammer & Schur, 1978) and probably macrophages (Mantovani,

Abbreviations: PEG = polyethylene glycol (av. mol. wt 6,000), ssDNA = single-stranded deoxyribonucleic acid, SDS-PAGE = polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate, EDTA = ethylenediamine tetracetic acid, CFD = complement fixation diluent, CFD/Ca^{++} = CFD containing 5 μl/ml 40% $CaCl_2:6H_2O$, CFD/EDTA = CFD containing 10 mM EDTA, PBS = phosphate-buffered saline, PMMA = polymethyl methacrylate, PMSF = phenyl methyl sulphonyl fluoride, PMMA-K = conglutinin linked to polymethyl methacrylate, NHS = normal human serum.

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Rabinovitch & Nussenzweig, 1975). Thus, since the complement-fixing complexes may well play an important role in the pathogenesis of rheumatoid arthritis, we decided to examine complexes isolated by adsorption to solid-phase bovine conglutinin columns through the calcium-dependent binding of complexed C3bi (cf. Casali & Lambert, 1979). The complexes were eluted in EDTA and analysed by previously established techniques. In addition, we have assessed the recovery characteristics of the conglutinin columns so that we could usefully compare the complexes isolated by the two different methods.

MATERIALS AND METHODS

Synovial fluids. Samples were obtained following therapeutic drainage of the knee joints of patients with definite or classical rheumatoid arthritis (ARA criteria), osteoarthritis, or traumatic arthritis who were attending the Middlesex Hospital Rheumatology Department. The fluids were spun to remove cells and larger debris and then either used immediately or stored at −20°C until required.

Bovine conglutinin. This was prepared by a method based on that of Lachmann & Hobart (1978). Briefly, 1 litre of raw ox serum with a conglutinating titre > 1/500 was heat-inactivated at 56°C for 30 min, calcified by the addition of 5μl/ml 40% $CaCl_2:6H_2O$ and incubated with 230 ml of a 50% suspension of sterilized baker's yeast for 1 hr at 4°C. The conglutinated cells were washed three times in 10 vol of CFD/Ca^{++} and the conglutinin eluted in PBS containing 10 mM EDTA, pH 7·2. The eluate was adjusted to pH 5·4 with orthophosphoric acid and dialysed into 0·01 M phosphate, pH 5·4. The precipitate thus formed was harvested, dissolved in CFD containing 0·1 M 2-mercaptoethanol for 1 hr at 4°C and finally dialysed into CFD. Insoluble material was spun out at 100,000 ***g*** for 30 min. This preparation gave a single precipitin arc when run against a rabbit antiserum to the yeast cell eluate.

Conglutinin columns were prepared by mixing a solution of bovine conglutinin with a titre of 10^7 and PMMA beads in a proportion of 375 μl to each gram of PMMA. The pH was raised to 9·5 with 0·2 M glycine/NaOH and stirred at 4°C for 3 hr. The beads developed maximum functional binding capacity under these conditions, as assessed by yeast cell rosetting. The beads were washed thoroughly with CFD, incubated with a blocking solution of 1 mg/ml bovine serum albumin for 15 min and washed again with CFD/Ca^{++} before packing in columns for use on the same day.

Isolation of complexes from synovial fluids. Aliquots of synovial fluids were treated with 50 iu/ml hyaluronidase at 37°C for 10 min to liquefy the fluids and facilitate their manipulation; they were then spun at 800 ***g*** for 15 min to remove fine debris. Frozen samples were alexinated (i.e. charged with C3bi) by incubation with an equal volume of fresh normal human serum diluted 1:2 in PBS for 7 min at 37°C. Where fresh synovial fluids were used, this step was omitted and in all other cases control experiments were performed to ensure that the activating sera contained negligible conglutinin-binding material. Proteolytic breakdown of complement components was inhibited by the addition of 100 mM PMSF to a final concentration of 2·5 mM. A fraction enriched with respect to immune complexes was prepared by adding 20% PEG in CFD to the synovial fluid (250 μl/ml) to give a final concentration of 4% PEG. Precipitation was encouraged by chilling on ice for 20 min and the precipitate so formed was spun down and redissolved in CFD/Ca^{++} at twice the original concentration. This step served the dual function of separating the complexes from complement fragments in the supernatant, which interfered with complex binding, and facilitated transfer of the complexes into CFD/Ca^{++}. This medium was more suitable for conglutinin binding, and gave better flow rates on the columns than synovial fluids applied directly.

The complex-rich fractions were applied to conglutinin columns which were then washed through with 3 column volumes (10 interstitial volumes) of CFD/Ca^{++}. Bound complexes were eluted by the addition of CFD/EDTA and fractions of 0·1 column volume were collected. Usually complexes emerged in fractions 4–9. The presence of complexes was determined by rocket electrophoresis of the eluted fractions against an anti-C3c.

In preliminary experiments, unlabelled human IgG aggregates were treated with NHS diluted 1:2 as previously described and applied at a range of concentrations to 1-ml conglutinin columns.

The bound aggregates were eluted in CFD/EDTA and the C3 content of the complexes was measured by rocket electrophoresis. Analysis of the data from this experiment indicated that the columns were saturated at a concentration of 20–30 μg C3bi/ml of PMMA-K. Furthermore, within the range 0–10 μg C3bi/ml PMMA-K the slope of the plot aggregates recovered/aggregates applied was linear and equal to 1·035, suggesting that non-specific losses onto the columns were negligible. In all subsequent experiments the binding capacity of the columns was not exceeded, i.e. in the range 0–10 μg C3bi/ml of column. It was also demonstrated that >98% of the potentially binding complexes were removed by a single passage down a PMMA-K column.

The characteristics of the columns were determined using ^{125}I-labelled IgG heat aggregates or transferrin/anti-transferrin complexes. Human IgG (5 mg/ml) was kept at 63°C for 10 min and the resulting aggregates labelled with radioiodine to a specific activity of 0·5 μCi/μg. ^{125}I-IgG aggregates were separated into fractions of defined mean sizes by sucrose density-gradient ultracentrifugation, and to each fraction was added a large excess of unlabelled aggregates of size range 7–25S to provide carrier protein. The fractions (0·5 ml containing aggregates at a final concentration of approximately 500 μg/ml) were alexinated with 0·5 ml NHS diluted 1:2 in PBS as above, precipitated with 4% PEG and isolated on the conglutinin columns. Recovery was quantitated by gamma counting. The recovery at each step for the different sized aggregates is shown in Table 1.

Recovery of complexes was estimated similarly as follows. Transferrin/anti-transferrin complexes were prepared in slight antigen excess by the addition of 200 μl rabbit anti-human transferrin to 1 ml of 12% human serum in PBS. Control samples to which 200 μl of PBS were added contained identical quantities of transferrin but no complexed transferrin. Sucrose density-gradient analysis indicated that the samples contained 48 μg complexed transferrin (>11S), equivalent to 28·6% of the total transferrin in the sample. The samples were precipitated with PEG and applied to conglutinin columns. Transferrin was estimated in the EDTA-eluted fractions to be 3 μg—a recovery of 6·3% similar to that obtained for the 25S aggregates. Control experiments showed that accurate quantitation of transferrin in the complexes by rocket electrophoresis was not markedly affected by the presence of the complexing antibody. No transferrin was detectable in the EDTA eluates of the control columns with serum only.

Analysis of immune complexes. Previously established techniques were used. Rocket electrophoresis and counter-current electrophoresis were carried out by the methods described by Weeke (1973). Isolated complexes were radiolabelled by the chloramine T method (Hudson & Hay, 1976). One-dimensional SDS-PAGE was performed using the system of Laemmli (1970) while two-dimensional SDS-PAGE was based on a modification of this method which has been described previously (Male & Roitt, 1979). Individual components were identified from the molecular weight of the parent molecule and the peptides derived from it, and by autoradiographs of SDS gels run on immunoprecipitates formed by counter-current electrophoresis and other agar gel techniques using radiolabelled complexes and antisera of known specificity (Male & Roitt, 1979).

Table 1. Recovery of IgG aggregates by conglutinin columns

	7S	14S	19S	25S
PEG supernatant	88·6	40·0	31·9	13·5
PEG precipitate	11·4	60·0	68·1	86·5
PMMA-K unbound	11·3	59·0	67·3	79·3
PMMA-K bound/eluted	0·1	0·9	0·8	7·2

Labelled aggregates of defined mean size were isolated by PEG precipitation and affinity chromatography on conglutinin columns. The percentage recovery of aggregates of each size is indicated for the successive steps of the purification procedure.

Antisera to the human proteins IgG, IgM, C3, C4, C5, C1q and factor B were obtained from Behringwerke (Hoechst, UK), to C3d from the Dutch Blood Transfusion Centre (Dienst, Holland) and to α_2-macroglobulin from Dakopatts (Mercia Brocades, UK). Antisera to $C\bar{1}r$ and $C\bar{1}s$ were a generous gift from Dr K. Reid and anti-IgA was kindly supplied by Dr G. Bull.

RESULTS

Complexes were isolated from the synovial fluids of 25 rheumatoid and seven ankylosing spondylitis joint effusions by affinity chromatography on PMMA-K columns. The recovery of complexes in each case was estimated by quantitation of C3 in the EDTA-eluted fractions by rocket electrophoresis against anti-C3. Summation of the amount recovered in the various fractions gave a measure of the total complement-fixing complexes in the synovial fluids. The recovery of complexes assessed by this method was significantly greater from the rheumatoid effusions ($3{\cdot}23 \pm 1{\cdot}79$ μg C3bi/ml of synovial fluid) than from ankylosing spondylitis effusions ($0{\cdot}76 \pm 0{\cdot}4$ μg C3bi/ml) at the 1% level of significance and from normal human serum ($0{\cdot}25 \pm 0{\cdot}10$ μg C3bi/ml) at the 0·1% level, by one-way analysis of variance. The normal human sera were identical to those used in the activation step. An additional experiment indicated that at least 98% of complexes capable of binding to the PMMA-K beads were removed on a single passage down the column.

In a preliminary analysis of the rheumatoid complexes by immuno-counter-current electrophoresis, C3 and C4 were detectable in all complexes while factor B was identified in 40%. Estimation of immunoglobulins by immuno-double-diffusion indicated the presence of IgM (80%), IgG (93%) and IgA (40%). It was noted that IgM was undetectable in complexes of three seronegative patients but present in complexes of all 12 seropositive individuals. This interesting finding was further investigated in a later series of experiments.

Complexes from 10 individuals were concentrated and analysed by two-dimensional SDS-PAGE. They were run without reduction in a 5% acrylamide gel which resolved proteins in the molecular weight range 60,000–500,000 daltons. The strips were then excised, reduced and applied across the top of 8·5% acrylamide gels giving resolution of peptides in the molecular weight range 20,000–200,000 daltons. Gels were fixed and the peptides visualized by staining with Coomassie blue. In this procedure, peptides from individual molecules are aligned in vertical groups. Examples of conglutinin-binding rheumatoid synovial fluid complexes analysed in this way are presented in

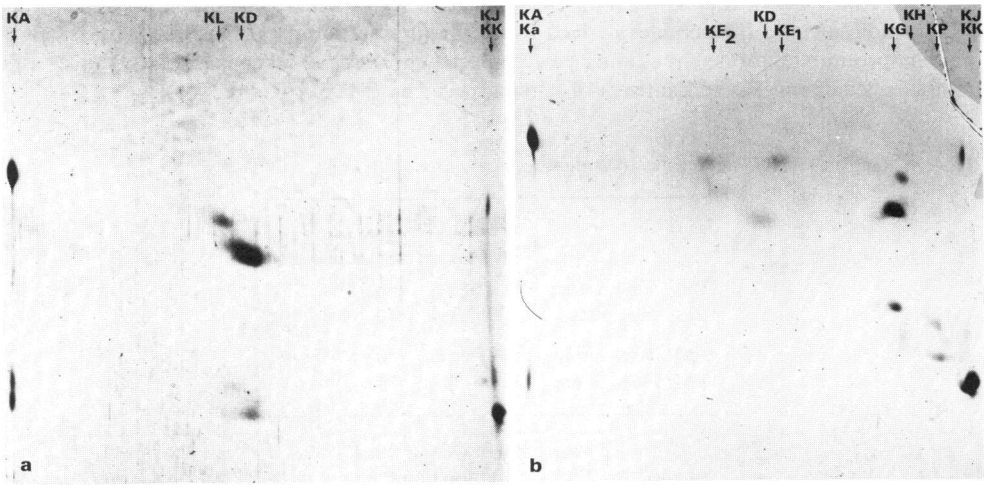

Fig. 1. Immune complexes from two patients isolated by conglutinin columns were separated in two-dimensional SDS-PAGE. Separation in the first dimension of proteins was from left to right. After reduction, peptides were resolved in the second dimension from top to bottom. Letter designations of peptide groups refer to Tables 2 and 3.

Table 2. Molecular components of conglutinin-binding rheumatoid synovial fluid complexes and their constitutent peptides

Group	Peptide mol. wt	Protein mol. wt	Identity	Relative abundance	Incidence* (%)
Ka	46,000	⩾500,000	C3 (α_4)	+	60
KA	78,000 26,000	⩾500,000	IgM	+++	100
KD	55,000 26,000	158,000	IgG	(+)-+++	100
KE_1	74,000 43,000 26,500	142,000	C3c	+-+++	40
KE_2	78,000 74,000 44,000	205,000	C3bi	(+)	80
KG	59,500 37,500	82,000	$C\bar{1}r$	+	50
KH	69,000 32,000	80,500	$C\bar{1}s$	+	50
KI	78,000	76,000	Bb	(+)	60
KJ	30,000 28,000 22,000	68,000	C1q	+	100
KK	26,500	66,000	C3d/C4′	(+)-++	100
KL	66,000 26,000	180,000	IgA	+	40

Proteins occurring in the majority of complexes of rheumatoid synovial fluids. Column 1 refers to two-dimensional SDS-PAGE groups. Molecular weights were derived by interpolation from standards in two-dimensional SDS-PAGE. Identities were determined by combined immunoelectrophoresis/SDS-PAGE. Incidence is the number of patients with detectable quantities of the molecule. Relative abundance is derived by visual estimation of Coomassie-blue-stained two-dimensional gels.

* Based on an analysis of 10 patients by two-dimensional SDS-PAGE.

Fig. 1a & b. The molecular weights of the peptides forming each group and the parent molecules they were derived from were calculated by interpolation from standards (thyroglobulin, fibrinogen, IgG, phosphorylase *a*, BSA, ovalbumin, DNase and Ig light chain). The results obtained by analysis of the fluids are given in Tables 2 and 3. The stoichiometry of the proteins may also be derived from this information. A great number of the peptide groups were identical to those identified in rheumatoid synovial fluid complexes isolated from sucrose/PEG gradients. To characterize individual molecules further, the complexes were labelled with ^{125}I and individual components precipitated in agar gels with specific antisera. The precipitin arcs so formed were washed extensively, excised and analysed in SDS-PAGE, the labelled peptides being visualized by autoradiography. Examples are shown in Fig. 2. Comparison of immunoprecipitation/SDS-PAGE

Table 3. Trace components of conglutinin-binding rheumatoid synovial fluids

Group	Peptides mol. wt	Protein mol. wt	Identity
KB	180,000 85,000	390,000	α_2-Macroglobulin
KC	64,000 52,000 45,000	285,000	?
KF	64,000 51,500	98,000	?
KP	32,000 36,500	67,000	?
KQ	83,000	69,000	?

Proteins occurring in less than 20% of rheumatoid synovial fluid complexes. Group and molecular weights were determined by two-dimensional SDS-PAGE. In one case, identity was established previously for sucrose/PEG-gradient-derived complexes. This analysis is based on 25 patients' complexes analysed by one-dimensional SDS-PAGE of which 10 were also analysed by two-dimensional SDS-PAGE.

patterns with two-dimensional SDS-PAGE groups made it possible to identify the majority of the proteins present.

There appeared to be two discrepancies between the data obtained by immunoprecipitation/SDS-PAGE and the two-dimensional SDS-PAGE groups. The immunoprecipitation/SDS-PAGE pattern obtained with anti-$C\bar{1}r$ did not correspond with any of the two-dimensional SDS-PAGE groups, but had the characteristic pattern of plasmin-cleaved $C\bar{1}r$. However, the group KG which invariably occurred in association with KH ($C\bar{1}s$) consisted of two of the components (mol. wt 37,500 and 82,000) present in the mixture resulting from plasmin cleavage of $C\bar{1}r$, and it would seem that the lack of correspondence between two-dimensional SDS-PAGE and immunoprecipitation/SDS-PAGE must be attributed to limited proteolysis of the $C\bar{1}r$ heavy peptide during the extended period of time required for the latter technique. The second discrepancy was the pattern produced by C4; in contrast to sucrose/PEG gradient complexes where C4b was invariably detected, one of the antisera to C4 precipitated a molecule from the conglutinin-binding complexes containing peptides of molecular weights 25,000 and 19,000. These correspond exactly to the α_3 and α_4 peptides formed by cleavage of the C4α chain with C3b inactivator (Fujita *et al.*, 1978). However, the lack of any C4β or γ chains indicated that there must have been some considerable dissociation or proteolysis during processing. It is also possible that the missing fragments were bound firmly to immunoglobulins and were not dissociated by the counter-current electrophoresis. It appears, therefore, that KK contains peptides of the C4α chain as well as C3d.

An additional observation was that the autoradiographic pattern of an anti-C3 precipitate in two-dimensional electrophoresis showed peptides of C3 in association with those of IgM; that is to say, material excluded from the 5% gel in the first dimension, as well as fragments corresponding to C3bi, C3c and C3d. This high-molecular-weight C3 yielded the α_4 peptide on reduction, the β chain lying beneath the IgM μ chain and a high-molecular-weight fragment ($\geqslant$200,000). This is evidence for covalent binding of C3b to the IgM molecule, presumably by the mechanism elucidated by Law & Levine (1977). There was, however, no evidence for such a covalent interaction with IgG. It also

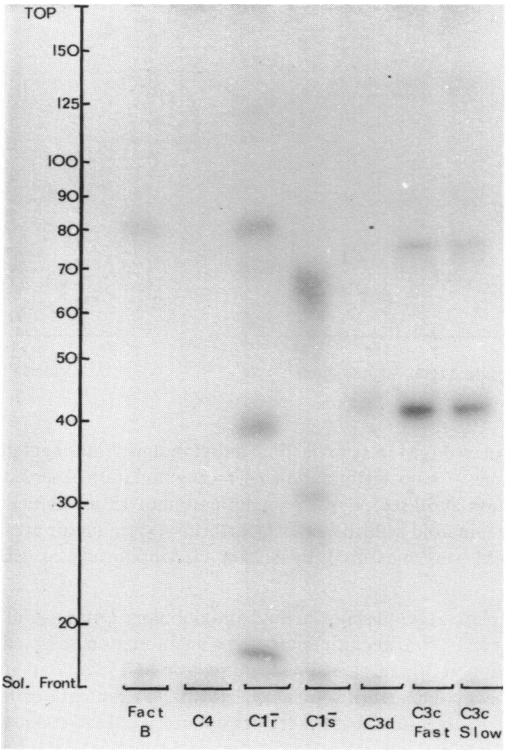

Fig. 2. Individual components of complexes visualized by autoradiography of one-dimensional SDS-PAGE of immunoprecipitates produced with antisera of the specificity indicated. Values show molecular weights of peptides in kilodaltons.

appeared that complexed C3 had variable mobility in counter-current electrophoresis; nevertheless, both fast and slow migrating C3 had the same peptide pattern in counter-current electrophoresis/SDS-PAGE analysis (Fig. 2).

It proved possible to identify all the protein components which were present with some regularity (a frequency of 40% of more) and from Table 2 it may be seen that all are immunoglobulins or activated complement components. Alpha-2-macroglobulin was identified as a trace component in one of 10 rheumatoid synovial samples analysed while four other components, which in each case constituted visually a small minority of the proteins, were only found in one or two fluids (Table 3). These results are compatible with the hypothesis that the complement-fixing complexes consist essentially of IgG and IgM antiglobulin and a variety of activated and inactivated complement components.

To test this hypothesis further, and following the interesting observation of an association between seropositivity and the incidence of complexed IgM, it was decided to compare synovial fluid antiglobulin titres with the levels of immunoglobulins in the conglutinin-binding complexes.

The first 35 available synovial fluids were processed and complexes were isolated by PMMA-K columns. The eluted fractions were tested for the presence of immunoglobulins G and M by rocket electrophoresis into gels containing carbamylated IgG of net zero mobility, with specificity for the appropriate immunoglobulin. Total complexed IgG and IgM was derived by summation of the quantities in each fraction and recovery was expressed as μg complexed IgG or IgM per ml of synovial fluid. Synovial fluid antiglobulins were assessed by latex agglutination and the RA haemagglutination test (RAHA). Before RAHA titres were measured, the fluids were absorbed three times with unsensitized sheep erythrocytes, which served to remove all activity against the unsensitized control cells in all cases but one which was excluded from the analysis. The relationship

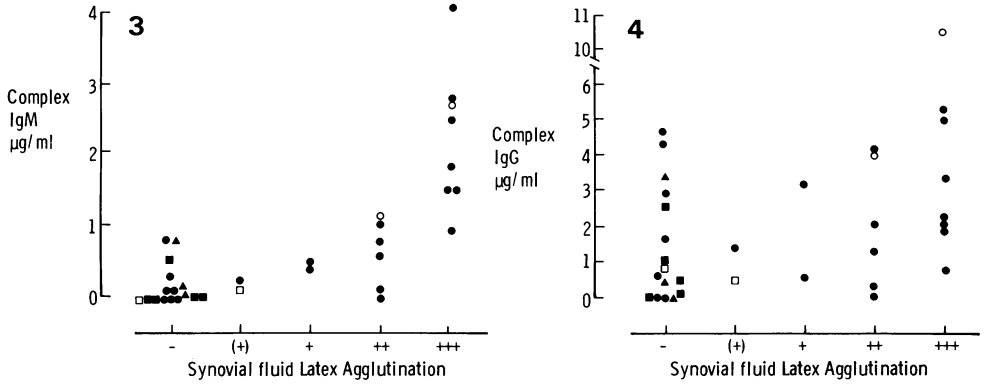

Fig. 3. Relationship of complexed IgM to synovial fluid antiglobulins. Latex agglutination of synovial fluid was assessed by agglutination time: − = no agglutination, (+) = agglutination observed at 5 min, + = agglutination at 1 min, ++ = agglutination at 30 sec, +++ = agglutination at 15 sec. Patient diagnoses: (•) rheumatoid arthritis, (○) palindromic rheumatoid arthritis, (■) osteoarthritis, (▲) traumatic arthritis, (□) other inflammatory arthritides. Complexed IgM was measured by rocket electrophoresis of EDTA-eluted fractions from conglutinin columns.

Fig. 4. Relationship of complexed IgG to synovial fluid antiglobulins. Latex agglutination of synovial fluid was assessed by agglutination time: − = no agglutination, (+) = agglutination observed at 5 min, + = agglutination at 1 min, ++ = agglutination at 30 sec, +++ = agglutination at 15 sec. Patient diagnoses: (•) rheumatoid arthritis, (○) palindromic rheumatoid arthritis, (■) osteoarthritis, (▲) traumatic arthritis, (□) other inflammatory arthritides. Complexed IgG was measured by rocket electrophoresis of EDTA-eluted fractions from conglutinin columns.

of complexed IgM and IgG to synovial fluid latex agglutination is indicated in Figs 3 & 4. It is evident that the presence of IgM in the complexes is closely related to latex agglutination titre ($r = 0{\cdot}815$; $P < 0{\cdot}001$; Spearman's rank correlation) whereas the association with complexed IgG was not so marked, since several patients with considerable levels of complexed IgG had negative latex titres ($r = 0{\cdot}448$; $P < 0{\cdot}01$). The relationship between antiglobulins and complexed IgM ($r = 0{\cdot}615$; $P < 0{\cdot}001$) or IgG ($r = 0{\cdot}370$; $P < 0{\cdot}05$) was also less marked when the rheumatoid factors were measured by RAHA assay. The sensitizing antigen in this assay is rabbit IgG as opposed to human IgG in the latex agglutination assay, and this difference may account for the weaker association observed. There was no significant correlation between synovial fluid anti-DNA titre, or total immunoglobulin of either class, and the levels of complexed immunoglobulins.

DISCUSSION

The present study was aimed at providing further analysis of rheumatoid synovial fluid complexes. In contrast to the sucrose/PEG gradient method for isolating complexes, the conglutinin columns gave a much lower non-specific recovery and therefore had a higher specificity, especially for the larger complexes, although the recovery was lower, particularly with respect to complexes of intermediate size (10–20 S). Overall we were unable to achieve the high recoveries (of the order of 60%) reported by Casali & Lambert (1979). With this technique, only complexes carrying active C3 moieties were isolated so that the analysis of this material provided data complementary to that previously obtained by the analysis of sucrose/PEG gradient complexes. In spite of the differences between the two isolation methods, the composition was qualitatively similar but quantitatively different. In particular, the conglutinin-binding complexes contained relatively more C3b, C3d, IgM, $\overline{C1r}$ and $\overline{C1s}$ and relatively less IgG and C1q. Since complexes were isolated by binding to conglutinin, the increased incidence of C3 is not unexpected, but in addition, the data support the

contention that complexed IgM is particularly important in complement fixation. This accords with the findings of Ruddy & Austen (1970) who observed depressed CH50 in seropositive but not seronegative synovial fluids in comparison with degenerative joint disease effusion. Similarly, some of our previous data indicated that complexed C1q promotes recovery in the sucrose/PEG gradient, presumably due to its low PEG solubility.

The techniques used here could detect individual proteins constituting 1–2% w/w of the complexes. All major proteins were identified as immunoglobulin or complement components and this data together with the strong association of IgM antiglobulins and complexed IgM strongly supports the view that the antiglobulins provide the main complement-fixing antibody system in rheumatoid synovial fluid. Only a minority (20%) of samples contained trace components which could not be identified and these differed from one fluid to another. These techniques are not capable of detecting wholly carbohydrate, lipid or DNA antigens. In a previous paper we found that a minority of rheumatoid complexes contained 1–2% w/w DNA with a relative enrichment of anti-ssDNA but not anti-dsDNA in the complex by comparison with the 7S fraction. It has been argued by Bell and others (1975) that this amount of DNA could be responsible for significant complex formation, as a DNA antigen with many determinants need only constitute a small proportion by weight of the complex. However, the lack of association of anti-DNA with the complexed immunoglobulin in the present studies makes it appear unlikely that the DNA/anti-DNA system makes an important contribution to the formation of complexes. It cannot be excluded that another antigen may trigger the disease process, but that by the time most patients present with knee effusions, antiglobulins, probably including self-associating IgG antiglobulins in association with IgM rheumatoid factor, are necessary and sufficient for the continual production of complement-fixing complexes in rheumatoid synovial fluid. There is also an indication that the response to human IgG best reflects the ability of the antiglobulins to form complexes, while the antibodies to other species' IgG are perhaps less important, although they do appear to have greater diagnostic significance for the differentiation of rheumatoid arthritis from other conditions.

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