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IMMUNE COMPLEXES AND COMPLEMENT IN RHEUMATOID ARTHRITIS

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Summary

Immune complexes have been shown to occur frequently during rheumatoid arthritis. They have been found in blood, in the synovium and in other extravascular lesions. The recent development of methods for the quantitation of immune complexes provided new tools to evaluate the possible role of immune complexes in rheumatoid arthritis. Immune complexes which appear in synovial fluid are in higher concentration than in serum and have particular physicochemical properties. They likely result from a local formation in the synovium and seem to be directly involved in the generation of the local inflammation. High levels of circulating immune complexes are usually associated with the development of extra-articular vascular lesions.

One of the major biological activity of immune complexes is to activate the complement system. There is indeed evidence of complement activation in circulating blood as well as in synovial fluid in patients with rheumatoid arthritis. The presence and the concentration of complement breakdown products in these fluids correlates with the clinical activity. Therefore, the analysis of immune complexes and of complement components appears useful for diagnosis and follow-up, and for the understanding of the pathogenesis of the disease.

Résumé

Des complexes immuns apparaissent fréquemment chez les malades atteints de polyarthrite rhumatoïde. Ils ont été mis en évidence dans le sang, l'espace synovial et dans des lésions extravasculaires. Le développement de méthodes de dosage de complexes immuns a permis d'évaluer leur rôle dans la pathogénie de la maladie.

Les complexes immuns apparaissant dans le liquide synovial sont en concentration plus élevée que dans le sérum et présentent des propriétés physicochimiques particulières. Ils semblent résulter d'une formation locale dans le tissu synovial et sont susceptibles d'intervenir directement dans la persistance de l'inflammation articulaire. Des taux élevés de complexes immuns circulants sont généralement associés au développement de lésions vasculaires extra-articulaires. L'activation du système du complément par les complexes immuns est également mise en évidence dans le sang comme dans le liquide synovial au cours de la maladie. Les taux de produits de dégradation de certains composants du complément reflètent assez étroitement l'activité clinique. L'analyse des complexes immuns et du complément apporte donc des informations utiles pour le diagnostic et la surveillance de la maladie, ainsi que pour la compréhension de sa pathogénie.

The association of articular manifestations with serum sickness in man and in animals suggested the possible involvement of immune complexes in rheumatic diseases. Although arthralgias commonly occur during a variety of infectious diseases (e.g. viral hepatitis) and a number of more defined inflammatory articular diseases which have often been considered as a consequence of localization of immune complexes in the synovium, direct histological evidence of such tissue localization has been obtained in rheumatoid arthritis (ZVAIFLER, 1973) and in systemic lupus erythematosus (MIESCHER, PARONETTO and LAMBERT, 1976).

It is well known that the biological effects of immune complexes depend on their recognition by soluble receptors, e.g., C1q, and by receptor molecules on cell membranes. These effects will be influenced by the nature of the antigen, of the antibody and by their concentrations. However, *in vivo*, the fate of immune complexes will also be directly dependent on the site of their formation. On one hand, most of the immune complexes appearing in circulating blood are cleared rapidly by the mononuclear phagocytic system and particularly by Kupffer cells. Only small complexes may persist for some time in the blood of normal individuals. A very small proportion of these circulating immune complexes may escape the clearing mechanisms and localize in vessel walls or filtering membranes, such as in renal glomeruli or in the choroid plexus. On the other hand, immune complexes are frequently formed in extravascular spaces, particularly if the antigens involved are released from tissue structures or from infectious agents. Such extravascular immune complexes are not cleared as rapidly as circulating immune complexes even when they reach a relatively large size. They can induce a local inflammation and their clearance will require the migration of cells with phagocytic properties into the site of the lesion. Therefore, the occurrence of soluble immune

complexes in extravascular fluids, such as joint fluids, is at least of equal importance as their persistence in circulating blood.

The presence of immune complexes was suspected in rheumatoid synovium on the basis of several observations: intra-articular depletion of complement (see below); the presence of deposits of immunoglobulins, often associated with complement factors in the synovial tissue and in phagocytic cells from articular effusion (KAPLAN and VAUGHAN, 1959; FISH et al., 1966; RODMAN et al., 1967; BRANDT, CATHCART and COHEN, 1968; BONOMO et al., 1969; KINSELLA, BAUM and ZIFF, 1969; MUNTHE and NATVIG, 1969); cryoprecipitation of immunoglobulin aggregates and complement fractions in synovial fluid (ZVAIFLER, 1973); demonstration of a biologically active factor (BAF) capable of inducing histamine release from perfused guinea-pig lung (BAUMAL and BRODER, 1968). Furthermore, a material similar to immune complexes has been detected in synovial fluids, using precipitation in gel in presence of serum containing rheumatoid factors (HANNESTAD, 1967; AGNELLO and KUNKEL, 1970; WINCHESTER, KUNKEL and AGNELLO, 1971) or in presence of C1q (AGNELLO, WINCHESTER and KUNKEL, 1970). Such material had characteristics similar to aggregates of immunoglobulins in ultracentrifugation analysis, with sedimentation rates between 7s and 30s.

In sera from patients with rheumatoid arthritis, immune complexes have also been demonstrated by ultracentrifugation and by precipitation with monoclonal RF (FRANKLIN et al., 1957; CHODIERKER and TOMASI, 1963; SCHROHENLOHER, 1966; WINCHESTER, AGNELLO and KUNKEL, 1970).

The nature of the immune complexes in serum and synovial fluids is still incompletely defined. Although complexes of 19s RF with IgG and of 7s antiIgG with IgG have been identified (FRANKLIN et al., 1957; KUNKEL et al., 1961) their relative importance is unknown and the possibility that other antigen-antibody systems may be involved in RA remains open. Recently, POPE, TELLER and MANNIK (1975a, 1975b) demonstrated that some of these immune complexes sedimenting from 6.6s to 19s are the result of a self-association of IgG molecules which have a rheumatoid factor activity. This self-association allows for the formation of cyclic structures, and the smallest detectable molecular complex is a dimer (mol. wt. 292 000).

The vascular lesions associated with rheumatoid arthritis were shown to be closely linked with a deposition of immune complexes in vessel walls (ZVAIFLER, 1973). The recent development of methods allowing for a direct measurement of the concentration of immune complexes in circulating blood and in extravascular fluids, including synovial fluid, have provided new quantitative data for the investigation of immune complexes in rheumatoid arthritis.

The detection of immune complexes in rheumatoid arthritis

A variety of methods have been recently developed for the quantitation of immune complexes in biological fluids (ZUBLER and LAMBERT, 1978). Some methods allow for a selective detection of immune complexes involving one given antigen through the discrimination between free and antibody-bound antigens. They are limited by a very restricted specificity. Most of the methods which are widely used have been devised in order to detect complexes regardless of the nature of the antigen involved in the formation of these immune complexes. They are based on the distinct physical or biological properties of complexed immunoglobulin molecules as compared with free immunoglobulins.

The formation of immune complexes leads to the occurrence of new molecular structures characterized by an increased molecular size, by changes in the surface properties, solubility and electric charge as compared with the corresponding free antigens and antibodies. These physical changes can be detected by ultracentrifugation analysis, by gel filtration or by selective precipitation. Unfortunately, these methods are limited by their lack of specificity and they are not adapted to routine investigations. Along these lines, cryoglobulins may often represent a particular type of immune complex but other proteins may also frequently be involved. The precipitation of immune complexes in polyethylene-glycol (PEG) is another example of the decreased solubility of complexes in well defined physico-chemical conditions. This property has been used to detect soluble complexes, but it should be considered as a rather non-specific screening test. Therefore, at the present time, none of the described physico-chemical methods seem suitable for the detection of immune complexes in routine clinical investigation.

Most of the detection of soluble immune complexes has been done using methods based on their biological recognition by humoral factors or by cellular receptors. Indeed, immune complexes can bind to the first component of complement, particularly C1q, and may trigger the activation of the complement system. They can also react with antiglobulins, e.g., rheumatoid factors, and if they are coated with C3 fragments, with immunoconglutinin or bovine congutinin. At the surface of many cells, immune complexes can bind to so-called Fc receptors which display a high avidity for aggregated or complexed immunoglobulins. They can also react with complement-receptors if C3 fragments are fixed to the complexes. The first method permitting a direct detection of immune complexes was described by AGNELLO, WINCHESTER and KUNKEL (1970). It was based on the precipitation of immune complexes in presence of an excess of C1q. Such an assay was simple but unfortunately lacked sensitivity and was only qualitative. In 1974, a quantitative assay, the C1q binding

Table 1. Principles of the biological assay for the detection of immune complexes

	Treatment of serum before testing	ab class in detectable IC	Risk of interference*	
			Positive	Negative
<u>Reaction with complement</u>				
Complement consumption e.g. anti-C activation C1q deviation	heat	G (1,3) M	b.p. PAS small inh.	
<u>In vitro complement binding e.g.</u>				
C1q binding assay	EDTA	G (1,3) M	PAS	b.p.
C1q solid-phase assay	EDTA	G (1,3)		C1q
<u>In vivo complement binding e.g.</u>				
conglutinin assay	-	G (1,3)		C3d
RAJI cell assay	-	G (1,3)	Ly ab	
<u>Reaction with Ig aggregates</u>				
Inhibition of anti-Ig e.g. monoclonal RF inhibition low avidity anti-Ig	dilute remove C1q + RF	G G (A?)	IgG IgG	RF, C1q
anti-ab	-	G		
<u>Binding to Fc receptors on cells, e.g. platelets aggregations leucocyte inhibition macrophage inhibition</u>				
e.g. platelets aggregations	-	G	HLA ab	RF, C1q
leucocyte inhibition	-	G	IgG, b.p.	
macrophage inhibition	-	G		RF

*b.p. = bacterial products; PAS = polyanionic substances; inh. = inhibitors; Ly ab = anti-lymphocyte antibodies; RF = rheumatoid factors; HLA ab = anti HLA antibodies.

test, was developed in our laboratory by NYDEGGER (1974) and was later modified by ZUBLER et al. (1976). This method was also based on the C1q binding activity of these complexes. Since that time, more than 20 methods have been described which measure the level of soluble immune complexes. The main methods are listed in table 1.

It should be expected that the specificity of each method devised for the detection of immune complexes varies according to the nature of immune complexes involved and to the relative influence of various interfering factors. It is also obvious that difficulty in standardizing some of the required reagents and the complexity of some of the proposed methods may hamper their applicability to routine laboratory investigations.

The clinical relevance of the measurement of immune complexes in rheumatoid arthritis should be considered at several levels: diagnosis, correlation with clinical features, follow-up during therapy, pathogenesis and aetiology.

With most methods, the incidence of immune complexes in synovial fluid appears relatively high in all types of RA patients, but elevated values can also be observed in other forms of inflammatory arthritis (GABRIEL and AGNELLO), 1977; ZUBLER et al., 1976b; MOHAMMED, THOMPSON and HOLBOROW, 1977). However, the incidence and the level of immune complexes in serum from these patients may vary considerably according to the method used. For example, when 24 serum samples from RA patients were analysed with 18 methods (LAMBERT et al., 1978) in several laboratories, the reported incidence of positivity was 83 per cent with the C1q-binding assay (C1q-BA), 71 per cent with the RAJI cell assay and the mRF-inhibition assay, and 42 per cent with the platelet aggregation assay. There is a significant correlation between the results obtained with these assays and this strongly suggests that the detected material is indeed related to immune complexes. However, it also emphasizes the fact that the immune complexes circulating in RA patients have limited biological activities, and cannot be similarly detected by methods based on the use of different biological recognition units.

The measurement of immune complexes may help in establishing an immunodiagnosis in patients with joint disease. It was found by ZUBLER et al. (1976b) that C1q binding material was detectable in relatively high concentrations in serum from patients with RA but not in degenerative, traumatic and even infectious arthritis (figure 1). However, one should be aware that circulating immune complexes are also present in other idiopathic inflammatory diseases, in some general infections, and often in cancer (ZUBLER and LAMBERT, 1978). There is a general agreement that immune complexes are more frequently detected and are present in higher concentrations in RA patients with extra-articular manifestations, and particularly with rheumatoid nodules (figure 2).

In most reported investigations, there was no correlation between the level of circulating immune complexes and the stage of RA or with the duration of illness. However, the C1q binding activity observed in serum during follow-up studies was closely associated with the clinical activity of the joint disease as assessed by classical clinical criteria (NYDEGGER et al., 1977).

Therefore, such measurement of immune complexes may be of interest for therapeutic trials where it would provide an objective feature, differing from other known biological parameters. Indeed, the concentration of immune complexes correlated neither with the erythrocyte sedimentation rate nor with the titre of rheumatic factor in seropositive patients. However, the incidence of immune complexes is higher in seropositive than in seronegative RA patients.

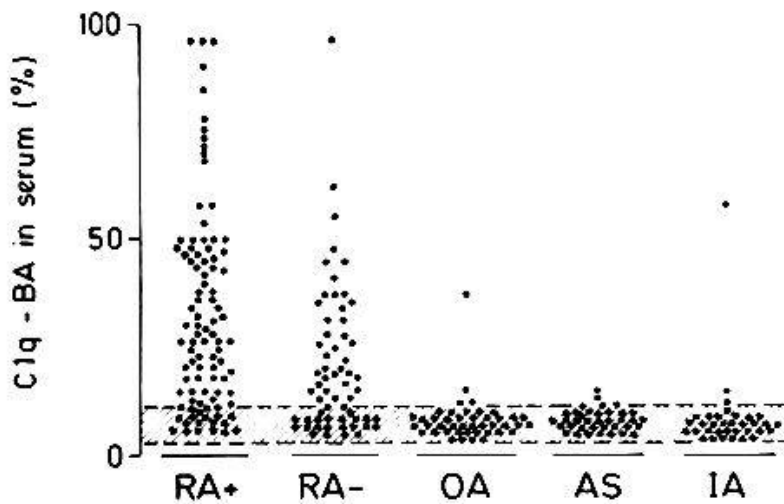


Fig. 1. Immune complex levels in serum samples (percentage ^{125}I -C1q binding activity, C1q BA) from patients with various joint diseases: seropositive (RA +) and seronegative (RA -) rheumatoid arthritis, osteoarthritis (OA), ankylosing spondylitis (AS), various other inflammatory arthritis (IA) (gout, chondrocalcinosis, infectious monoarthritis). The shadowed area represents the normal range (mean \pm 2 SD) of the values found in healthy blood donors.

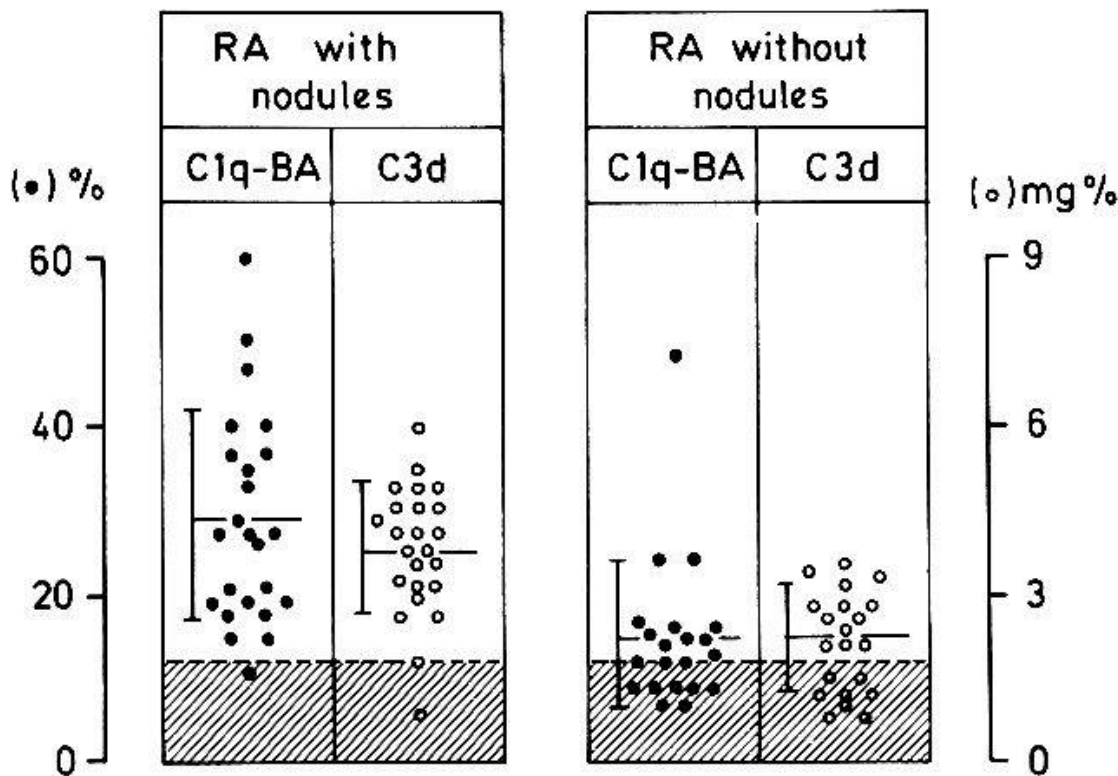


Fig. 2. Correlation of the immune complex levels in serum (percentage ^{125}I -C1q binding activity, C1q BA) and the C3d concentrations in plasma with the occurrence of subcutaneous nodules in patients with rheumatoid arthritis. The means of the values (\pm 2 SD) from each group are indicated. The shadowed area represents the normal range (mean \pm 2 SD) of the values observed in healthy blood donors. ● = C1q-BA %; ○ = C3d mg %.

The size of C1q binding immune complexes has also been characterized. Large C1q binding material can be found in synovial fluid but most of the serum immune complexes are smaller than 19s. They can be dissociated in acid with a release of 7s IgG (ZUBLER et al., 1976b). In ankylosing spondylitis, immune complexes can also be detected in serum from patients but the levels observed are much lower than in RA (GABAY et al., 1977).

Complement activation in rheumatoid arthritis

An involvement of the complement system in the pathogenesis of RA was suspected when it was observed that the total hemolytic complement activity and C2, C4 and C3 concentrations in joint fluids from patients with RA were significantly depressed as compared to levels measured in synovial fluids from patients with degenerative joint disease (FOSTIROPOULOS et al., 1965; HEDBERG, 1964; RUDDY and AUSTEN, 1970). Such abnormal complement profiles provided evidence for the presence of some factors causing depletion and utilization of complement proteins. The simultaneous presence of C3 and immunoglobulin deposits in synovium and in phagocytic cells of articular effusions from RA patients was suggestive of the presence of immune complexes. Whole hemolytic complement activity (CH50) is usually normal or elevated in serum from patients suffering from rheumatoid arthritis in the absence of systemic rheumatoid vasculitis (SCHUBERT et al., 1965), although a few RA patients have had episodes of hypocomplementemia, usually associated with an exacerbation of the disease (FRANCO and SCHUR, 1971). In contrast, the synovial fluid from patients with seropositive RA exhibits a decreased complement activity when compared to that measured in patients with osteoarthritis (PEKIN and ZVAIFLER, 1964).

Activation of the complement system proceeds through two pathways - the classical and the alternative. Both lead to the activation of C3 with the generation of C3b and C3a. C3b is involved in both the activation of the late-acting complement components C5 - C9 and the intrinsic activation of the alternative complement pathway, leading to an augmented formation of the "amplification" C3 convertase, C3b, Bb. During this process, factor \bar{D} cleaves factor B into a large fragment, Bb, and a small one, Ba. The participation of C3b is controlled by its cleavage into C3 and C3d through the combined action of 2 control proteins: C3b inactivator and β 1H.

In the classical complement pathway, activation of C1 promotes the generation of another C3 convertase, C42. During this sequence, C4 is also cleaved into C4a and C4b, which further decays to C4c and C4d. Therefore, the appearance in a biological fluid of fragments such as C3c, C3d, C4d or Ba suggests complement consumption.

The complement system in rheumatoid arthritis has been studied in synovial fluid by measuring the level of complement components by hemolytic or immunologic methods (PEKIN and ZVAIFLER, 1964; RUDDY and AUSTEN, 1970). Unfortunately, an increased synthetic rate can mask an increase in catabolism of these proteins and such static studies are of limited practical value for the study of this disease. Turnover studies performed *in vivo* with radio-labelled complement components allow for a better estimation of the catabolism of complement components. It was observed that patients with RA may display an increased catabolism and/or an increased synthesis of C3 (RUDDY et al., 1975). Recently, a hypercatabolism of C4, mostly in the extravascular space, and a hypercatabolism of factor B, were also reported in 15 RA subjects (KAPLAN et al., 1978).

An alternative approach to investigate the role of the complement system in various clinical conditions is to detect the presence of breakdown products of complement components in plasma or synovial fluid. Such products have been demonstrated in synovial fluids from patients with RA by means of analytic methods based on the change in physicochemical properties and in antigenic constitution of complement components occurring during complement activation.

Some methods have been developed to directly quantitate C3d, C4d and Ba fragments resulting from the breakdown of C3, C4 and factor B, respectively, and have also been applied to the investigation of synovial fluids (LAMBERT et al., 1975).

The mean level of C3d was very significantly higher in RA patients than in patients with osteoarthritis (OA). Generally, patients with low native C3 levels had greatly increased C3d levels. Although the level of factor B was normal or increased in synovial fluid from patients with RA, fragments of this protein, Ba, were found in relatively large amounts as compared to the OA levels. The mean level of synovial C4 was slightly lower in patients with RA than in OA (PERRIN et al., 1975). The C4d levels were significantly increased in RA. A significant correlation was found between the levels of C3d and those of Ba in individual RA patients but not between the levels of C3d and those of C4d. This suggests that the involvement of the amplification convertase in the hypercatabolism of C3 exceeds that of the classical C42 convertase.

The generally normal plasma levels of complement components encountered in RA have been explained by increased synthesis of complement components associated with the inflammatory syndrome in RA thus masking a possible increase in catabolism. Measurements of C3d in plasma from RA patients provided evidence that a systemic activation of C3 is likely. Indeed, C3d fragments were found to be elevated in 80% of 45 plasma samples (NYDEGGER et al., 1977), but the concentrations were lower than in synovial fluids (fig. 3). The quantitative analysis

of these data is consistent with systemic intra- or extra-vascular generation of the circulating C3d and excludes the hypothesis that the joint space is the only source of plasma C3d. So far, the characteristic pattern of the complement profile in RA, including a marked depression of C4 levels, was held to fully reflect activation of the complement system by immune complexes through the classical pathway.

The levels of immune complexes were compared to those of C1q, C4, C3 and B in single synovial fluid samples from RA patients. A significant inverse correlation was found between the level of immune complexes, expressed by C1q BA and the C4 levels, suggesting activation of the classical pathway by the complexes. Furthermore, the C1q BA levels in the blood and synovial fluids samples from RA patients were compared with those of C3d and it appeared that these two variables were significantly correlated in both the intravascular and the intraarticular compartments (fig. 3).

The dual role of the alternative complement pathway in initiation of complement activation and in the intrinsic regulation of the complement sequence is now well recognized. Because of the normal presence of \bar{D} , the interactions of the activating components of the alternative pathway must be equilibrated with regulatory proteins. Continuous low grade generation of C3b by the fluid phase interaction of purified native C3, B, P and \bar{D} is normally prevented from advancing to C3b-dependent amplified C3 cleavage by the control proteins, C3bINA and β 1H (reviewed in: FEARON et al., 1976). However, once amplification convertase C3b, Bb is formed, its spontaneous decay may be considerably retarded by properdin interacting with C3b to form the complex P, C3b, Bb, which is the stable and active form of the convertase. The combined actions of component proteins, which tend to assemble the amplification convertase, and of the control proteins, which tend to dissociate it, can be pictured as a balance, where even very modest modifications in the concentration of proteins on either side can disturb its equilibrium (NYDEGGER et al., 1978). Most of the quantitative data on alternative pathway proteins in RA have been collected so far by Whaley and Ruddy (WHALEY et al., 1978). In 13/20 (5/18) patients with seropositive and 4/9 (3/9) patients with seronegative RA supranormal serum level of C3bINA (or β 1H, numbers in brackets) were found and these values correlated well with C3 and B but not with C4 levels. This could mean that the immune complex-induced hypercatabolism of complement is dampened by an amplification pathway which is itself attenuated by its control proteins.

In synovial fluid both C3bINA and β 1H were depressed during seropositive RA. As previously suggested, local depression of C3bINA and β 1H levels in patients with RA may reflect decreased synthesis, increased catabolism, increased efflux or decreased influx from and into the joint space. The decreased levels of C3bINA and β 1H in synovial fluid could favor

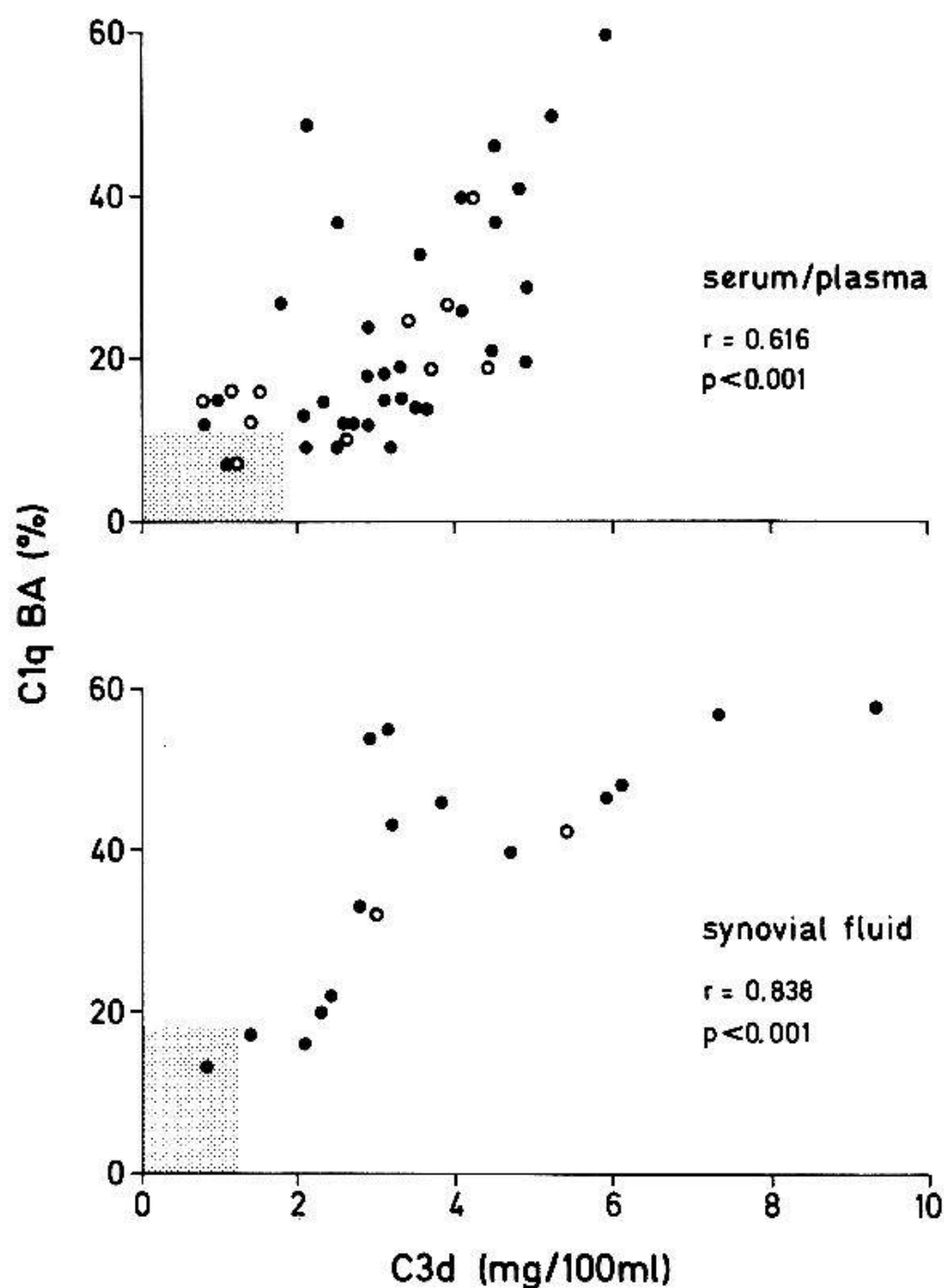


Fig. 3. Upper part: correlation between C3d levels in plasma and ^{125}I -C1q-BA in paired serum samples from patients with seropositive RA (RA +) (●) and seronegative RA (RA -) (○). Lower part: correlation between C3d levels and C1q BA in individual synovial fluid samples. The shadowed areas indicate mean + 2 SD limits of normal range as observed in patients with DJD and healthy blood donors.

an intense activation of the amplification pathway thus adding to the overall complement activation triggered by immune complexes via the classical pathway.

The possible involvement of complement in the pathogenesis of RA is suggested by the correlation observed between the clinical expression of the disease and some of the complement parameters.

Various laboratory and clinical parameters of RA were used to define more precisely the patient's actual clinical condition at the time the samples for analysis of C3d and C1q BA levels were obtained. The C3d level was significantly increased ($p < 0.001$) in patients with extraarticular symptoms, mostly nodules, as compared to non-nodular RA (fig. 2). Disease staging was available in 47 patients with consideration of further anatomical features of RA. There was a significant correlation between the C3d levels in plasma and the clinical activity of the disease (NYDEGGER et al., 1977).

Conclusions

There is good evidence that immune complexes occur frequently during rheumatoid arthritis. This evidence is based on the demonstration of immune complexes in blood, in various extravascular fluids and in some tissue structures.

The presence of soluble immune complexes has been suspected from physicochemical analysis of serum and synovial fluid, and from the recognition of biological effects largely specific for such immune reactants.

The recent development of methods allowing for a quantitation of immune complexes based on some of their biological reactivities, provides a new approach for the investigation of the pathogenesis of rheumatic diseases. In view of the data presently available, it appears that immune complexes may occur in a large number of pathological conditions and that their pathogenicity may be expressed at various degrees. Obviously, the biological activities of these naturally occurring immune complexes differ according to the clinical situation and this heterogeneity is responsible for the various types of reactivity observed in the biological assays used for the detection of immune complexes.

Although it is generally considered that circulating immune complexes play an essential role in the development of vascular lesions associated with rheumatoid arthritis, we think that the existence of a large extravascular pool of soluble and insoluble immune complexes should be considered in relation to the frequent occurrence of inflammatory lesions in the extravascular compartment. The possible pathogenic role of immune complexes formed in the extravascular spaces is particularly demonstrated in articular localization of rheumatoid arthritis. For example, it is clear that immune complexes are present in synovial fluid from

patients with rheumatoid arthritis in higher concentrations than in serum, and that their particular physicochemical characteristics are expressed in a particularly efficient biological activity. It is unlikely that such complexes would be the result of a simple diffusion of circulating immune complexes into the synovium. The persistence and the pathogenicity of immune complexes directly formed in the extravascular compartment are certainly favoured by the limited clearance capacity of the mononuclear-phagocytic system in this compartment as compared to the efficient clearance mechanism operating in the circulation and particularly during passage through the liver and spleen.

Activation of the complement system also most likely occurs both locally in joints and systemically, i.e. in circulating blood or at extraarticular localizations. Immune complex-induced activation of the classical pathway may represent the main and early pathogenic event which can, however, be regulated by the intervention of mechanisms such as the alternative pathway of complement as an amplifying or dampening effector system. The supranormal levels of the control proteins C3bINA and β 1H found in serum from RA patients suggest that excessive systemic complement activation is prevented in circulating blood. Conversely, in the synovial space, the low control protein levels would favor excessive complement consumption as demonstrated by extremely high levels of C3d and Ba fragments. The clinical relevance of the detection of immune complexes and of the evaluation of their effect on the complement system has been particularly demonstrated in rheumatoid arthritis. Such parameters appear useful for the diagnosis, follow-up during therapy, and understanding of the pathogenesis of clinical manifestations. One can hope that the present efforts to isolate and identify the components of the immune complexes will also provide information relevant to the aetiology of some of the rheumatic diseases.

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