Complement-fixing ability of antinuclear factors

Studies in adult and juvenile rheumatoid arthritis and systemic lupus erythematosus

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The presence of circulating antinuclear factors (ANF) in connective tissue diseases is well known.

Recent studies have shown differences in the complement (C')-fixing ability of ANF from patients with systemic lupus erythematosus (SLE) and a heterogeneous group of connective tissue diseases (Peltier, 1968; Brunner and Davis, 1970). In addition, a recent study has suggested that renal lesions in SLE is related to the C'-fixing ability of ANF (Tojo, Friou, and Spiegelberg, 1970).

Immune complexes formed by nuclear antigens and antinuclear antibodies, with fixation of C', are thought to be involved in the renal lesions of this disease (Koffler, Schur, and Kunkel, 1967; Cossio, Arana, and García Morteo, 1970). Potentially pathogenic immune complexes are formed by antibodies that are able to interact with C' (Ishizaka, Ishizaka, and Campbell, 1959).

Since ANF are frequently observed in rheumatoid arthritis (RA), which do not develop similar renal lesions, it was interesting to investigate the C'-fixing ability of ANF from patients with this disease in comparison with a group of patients with SLE.

Material and methods

21 patients with classical or definite RA according to the ARA criteria (Ropes, Bennett, Cobb, Jacox, and Jessar, 1959), and fifteen patients with juvenile RA (Bywaters, 1967) with ANF by indirect immunofluorescence in their sera, were selected. Sixteen SLE patients were also studied.

ANF were investigated by indirect immunofluorescence technique, using as substrate cryostat sections of mouse liver (Hamard, Cannat, and Seligmann, 1964). Observations were performed with a Leitz Ortholux microscope with dark field condensator, using an Osram lamp

HB0200 with UG1 filter. Goat antibody globulins, fluorescein-labelled, were obtained from a commercial source* for total human immunoglobulins, IgG and IgM, and were used at dilutions varying between 1 in 6 and 1 in 10. Fluorescein-labelled anti-human IgA was obtained through the kindness of Dr. Daniel Hurez† and was used at a dilution of 1 in 10. Antisera for immunoglobulin class was made monospecific for the G, A, or M chain by absorption with the other immunoglobulins: by immunoelectrophoresis (Figure) and double diffusion analysis they react giving one precipitin line.

C'-fixation by antinuclear factors was assayed by indirect immunofluorescence with inactivated patients' sera, using fresh normal human sera as a source of C' (Lachmann, Müller-Eberhard, Kunkel, and Paronetto, 1962), and fluorescein-labelled goat anti-human β_{1A-C} globulin* at a dilution of 1 in 5. The specificity of this antiserum was demonstrated by immunoelectrophoresis against fresh normal human serum; it reacted giving one precipitin line corresponding to β_{1A-C} (Figure).

Anti-human gammaglobulin factors were studied by the latex-fixation test (Singer and Plotz, 1956).

Results

ANF from eight (22 per cent.) of 36 patients with RA fixed C'. The incidence of C'-fixing ANF was similar in the adult (4 out of 21; 19 per cent.) and juvenile (4 out of 15; 26 per cent.) forms of RA. In contrast, fixation of C' was observed in fifteen (93 per cent.) of sixteen sera of patients with SLE.

* Hyland Division Travenol Laboratories. Fluorescein conjugated antiglobulins are prepared according to the specifications for the production of anti-human IgG conjugates set forth at the London Round Table Conference on Standardization in Immunofluorescence, October, 1968.

† Laboratoire d'Immunochimie, Institut de Recherches sur les Maladies du Sang, Paris. The antiserum was prepared by immunizing rabbits with human IgA myeloma proteins and labelled with fluorescein isothiocyanate according to the method of Nairn (1968). The antiserum was already specific for a chain.

FIGURE Immunoelectrophoresis of antisera monospecific for G (IgG), A (IgA), or M (IgM) chain and 1A-C globulin
NHS = Normal human serum

Table I Complement-fixing ANF of RA and SLE patients related to titres

Diagnosis	No. of cases	ANF		Total C'-fixing		
		Titre	No. of cases	C'-fixing	No.	Per cent.
RA	36*	<1 in 50	30	7	8	22
		≥1 in 100	6	1		
SLE	16	<1 in 50	7	6	15	93
		≥1 in 100	9	9		

* 21 adult + 15 juvenile.
† 4 adult + 4 juvenile.

The difference between RA and SLE groups was significant (P < 0.001).

In order to evaluate if ANF titre was related to C'-fixing ability, samples were grouped according to their final titre, (Table I): seven of thirty RA with titres < 1 in 50, and one of six with titres > 1 in 100 fixed C'. Six out of seven SLE with titres < 1 in 50 and all of nine with titres ≥ 1 in 100 also fixed C'. Thus, the titre was not responsible for the difference.

The immunoglobulin class of ANF in twenty RA patients was also studied (Table II overleaf). With some exceptions, ANF activity was present in more than one of the immunoglobulins investigated. ANF in the IgG class were found in almost all sera and this was the only immunoglobulin with nuclear reactivity in the three sera of the group not fixing C'. Anti-nuclear activity was found only in IgM in three cases, one of which fixed C'.

Although IgM differences between both groups

were not statistically significant, it is of interest that IgM ANF was observed in only four of fifteen patients with antibodies not fixing C' and four of five patients with C'-fixing ability.

Table II *Immunoglobulin class of ANF in twenty RA patients*

<i>C'</i> -fixation	Case no.	<i>Immunoglobulin</i>			
		<i>G</i>	<i>A</i>	<i>M</i>	
Negative	1	+	+	—	
	2	—	—	+	
	3	+	+	—	
	4	—	+	—	
	5	+	+	—	
	6	—	—	+	
	7	—	+	+	
	8	+	—	—	
	9	+	+	+	
	10	+	+	—	
	11	+	+	—	
	12	+	—	—	
	13	+	+	—	
	14	+	+	—	
	15	+	—	—	
	Total	15	11	10	4
Positive	16	+	+	+	
	17	+	+	—	
	18	+	—	+	
	19	—	+	+	
	20	—	—	+	
	Total	5	3	3	4

C'-fixation was not related to the presence of rheumatoid factors (RF) in sera. As seen in Table III, ANF with C'-fixing ability were present in four of seventeen sera with RF, and four of nineteen without.

Table III *Complement-fixing ANF of RA patients related to anti-gammaglobulin factors*

Serology	No. of patients	C'-fixing
Seropositive	17	4
Seronegative	19	4

The presence of possible inhibitors of C'-fixation in some rheumatoid sera was also studied. Each of three SLE sera with C'-fixing ANF were diluted (1 in 10) in three RA sera with C' not fixing ANF and high titres of RF. The mixtures were incubated for 1 hr at 37°C. and overnight at 4°C. Controls were the same lupus sera diluted in inactivated normal human sera, and isotonic saline. C'-fixation by ANF of the SLE sera remained unaffected, and further dilutions showed that the C'-fixing end titre was the same as in the controls.

Discussion

This report shows a difference in the complement-fixing ability of antinuclear antibodies from RA and SLE patients. The lack of C'-fixation in the RA group was not related to the titre, or to the immunoglobulin class of ANF; moreover in a great number of patients the immunoglobulins belonged to the IgG class which usually fixes C'. Although there are differences in C' affinity between subclasses of IgG, it has been shown that, at least in SLE, ANF are similarly distributed among them (Tojo and Friou, 1968; Tojo and others, 1970). The results of this work are similar to those reported recently in patients with Felty's syndrome (Brunner and Davis, 1970).

The incidence of C'-fixing ANF was similar in the adult (19 per cent.) and juvenile (26 per cent.) forms of RA. No correlation between C'-fixing ability and extra-articular involvement was found: the only three RA patients with systemic involvement (Sjögren's syndrome, necrotizing arteritis, and iridocyclitis) had no C'-fixing antibodies.

Previous works have suggested that rheumatoid factor may inhibit C'-fixation of some antigen-antibody systems (Zvaifler and Bloch, 1962; Heimer, Levin, and Kahn, 1963; Gough and Davis, 1966). In this study a similar incidence of C'-fixing ANF was observed in the seropositive and seronegative groups. Furthermore, the end titre of C'-fixation by antinuclear antibodies of SLE was not modified when diluted in sera with high titres of rheumatoid factor and C' not fixing ANF. These results suggest that the C'-fixing ability of ANF is not influenced by antigammaglobulins or other serum inhibitors.

The present work suggests that the difference in C'-fixing capacity of ANF from RA and SLE sera may be of diagnostic value. Further follow-up of RA patients with C'-fixing ANF would be of interest in order to establish the prognostic value of this finding. In this regard, it is of interest that after 1 year one patient with classical RA and C'-fixing ANF developed the complete serological syndrome of SLE with cutaneous involvement typical of scleroderma.

Since immune complexes involving ANF have been related to lupus nephritis (Koffler and others, 1967; Cossio and others, 1970), and C'-fixation is essential for tissue damage, it could be postulated that the absence of renal lesions in rheumatoid arthritis patients with ANF could be partially explained by their lack of C'-fixing ability. However, other factors concerning the pathogenic properties of immune complexes should be taken into consideration (Pincus, Haberkern, and Christian, 1968).

Summary

Data are presented on the complement-fixing ability of antinuclear factors from the sera of 36 patients, 21

with adult and fifteen with juvenile rheumatoid arthritis; sixteen with systemic lupus erythematosus were also studied. Eight (22 per cent.) of the 36 rheumatoid patients and fifteen (93 per cent.) of the sixteen patients with SLE had complement-fixing antinuclear antibodies. The difference between the groups is statistically significant ($P < 0.001$).

The incidence of complement-fixing antinuclear antibodies in patients with adult and juvenile forms of rheumatoid arthritis was similar.

In this study, the complement-fixing ability of antinuclear antibodies in rheumatoid arthritis was not related to titres of antibodies, immunoglobulin class of antinuclear factors, presence of serum inhibitors, or antigammaglobulin factors. The possibility is raised that a study of this property of

antinuclear antibodies in patients with rheumatoid arthritis and systemic lupus erythematosus may be of diagnostic value.

Since immune complexes involving antinuclear factors have been related to lupus nephritis, and complement-fixation is essential for tissue damage, it could be postulated that the absence of renal lesions in rheumatoid arthritis patients with antinuclear factors could be partially explained by an absence of ability to fix complement.

We are grateful to Dr. Daniel Hurez for the IgA antiserum, to Miss Corina Zavalia and Miss Coca Casanova for valuable technical assistance, and to Miss Brigid Lewis and Miss Elisa T. Meyer for the revision of the English manuscript. This work was supported in part by grants from ADAR and Fundación Cossio.

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