Complexed rheumatoid factor measurements in sera, synovial fluids and in immune complex fractions[1]

A. Falus, Katalin Merétey, Ute Böhm and S. Bozsóky[2]

Department of Rheumatology and Physiotherapy, Postgraduate Medical School, Budapest, and National Institute of Rheumatology and Physiotherapy, P.O.Box 54, H-1525 Budapest 114 (Hungary), 6 June 1978

Summary. Mild acidic treatment increases the rheumatoid factor titre of some sera and synovial fluids (SF) in rheumatoid arthritis (RA), juvenile RA (JRA) and most frequently in rheumatoid vasculitis. This unmasking of 'hidden' RF in serum and SF samples correlated with the RF-immune complexes (RF-IC) and complexed C4 present in the 3% polyethylene glycol (PEG) precipitates, indicating that by means of 'hidden' RF measurements RF-ICs are possibly detected. This method seems to provide a diagnostic tool for detecting RF-ICs in RA and other related diseases.

Various types of immune complexes play a definite role in the pathogenesis and maintenance of different inflammatory joint diseases[3,4]. Within the broad spectrum of ICs in RA patients, the frequent occurrence of complexed 7S and 19S anti-IgG autoantibodies (RF) may indicate the significance of rheumatoid factor containing ICs of either intermediate or 22S size[5,6]. 'Hidden' form of RF (not detectable by the conventional serological methods) was found by means of gel filtration and radioimmune methods in RA patients[7], patients with nodular seronegative polyarthritis[8], JRA cases[9] and in healthy persons[10].

In this paper we describe a simple method for the detection of 'hidden' RF, evaluating the differences in RF titres after an incubation at neutral and acidic pH, respectively.

Materials and methods. 370 sera and 172 synovial fluids from RA patients (according to the diagnostic criteria of ARA) and from rheumatoid vasculitis, JRA, osteoarthrosis (OA) and ankylosing spondylitis (AS) cases were examined. Sera and digested SFs (15 IU/ml hyarulonidase, Hyason, at 37 °C for 1 h) were inactivated at 56 °C for 30 min. Aliquots were 4-fold diluted either with 0.1 M phosphate buffered saline (PBS, pH 7.4) or with 0.1 M citrate buffer (pH 3.9), and incubated overnight at 4 °C. The RF titres of diluted samples were determined by microplate agglutination of rabbit IgG sensitized sheep red blood cells (Rose-Waaler system), in 2 parallels. Neutralization of acidified samples was done in the 1st row of microplates by means of 0.4 M disodium phosphate solution, immediately before titration. An at least 4-fold increase in RF titres after acidic treatment was accepted as criterion for positivity of 'hidden' RF.

Immune complex enriched fractions from native serum and SF samples were isolated by precipitation with 3% PEG 6000 (Fluka) according to Creighton et al.[11]. Samples (0.3 ml) were diluted 1:25 in 0.1 M borate buffer (pH 8.2) and mixed thoroughly with equal volume of 6% PEG. After 18 h incubation at 4 °C and centrifugation (3000 × g for 20 min) pellets were washed three times with 3% PEG solution and finally dissolved in 0.3 ml PBS. RF titres were measured in IC fractions after acidification as described above. Protein content was controlled by Lowry's method. Level of 4th complement component (C4) was determined in PEG fractions by means of single immunodiffusion plates using specific antiserum[12].

Results and discussion. Frequency of the presence of 'hidden' RFs in sera and SFs of different joint diseases is summarized in table 1. 'Hidden' RF in sera of RA (36%) and JRA (26%) patients occurred more often than in OA (10%) and AS (16%) cases. Very high frequency of masked RF was detected in the sera of patients with rheumatoid vasculitis (88%). In SFs, 'hidden' RFs were found in higher percentage than in sera of the same group. Higher incidence of 'hidden' RFs in seronegative than in seropositive cases can be clearly seen. An 8-fold or even higher increase in titre was found in some patients, mostly in vasculitis cases.

Parallel study of the native samples and corresponding IC-fractions showed (table 2) that in the majority of the 'hidden' RF positive serum and SF samples, their 3% PEG-insoluble fractions also contained acid-dissociable RF-ICs. The lack of masked RF in IC fractions and native samples without 'hidden' RF was also often observed. In a minority of the results, discrepancy was observed. We failed to detect 'hidden' RF in 10 sera and 7 SFs, but complexed RF antibodies were found in their PEG-insoluble fractions. Either inhibition of (unmasked) RFs by excess of monomeric IgG or dissociation of RF-complexes even in neutral buffer can be considered. When 3% PEG precipitation is performed, monomeric IgG molecules are avoided and stabilization of non-avid complexes may be produced by PEG.

In 'hidden' RF positive samples without detectable PEG-insoluble RF (4 sera and 4 SF) only a suggestion can be

Table 1. Detection of 'hidden' RF in sera and synovial fluids of seronegative and seropositive patients

Group	Sample	Number of cases (n)	'Hidden' RF positive		Seropositive* 'hidden' RF			Seronegative** 'hidden' RF		
			n	%	n	positive n	%	n	positive n	%
RA***	Serum	231	82	36	138	43	31	93	39	43
	SF	129	60	47	60	22	37	69	38	55
JRA	Serum	74	19	26	12	2	17	62	17	27
	SF	8	3		0	0		8	3	
Rheumatoid vasculitis	Serum	16	14	88	14	12	86	2	2	
OA	Serum	30	3	10	2	0		28	3	12
	SF	18	2	11	1	0		17	2	12
AS****	Serum	19	3	16	3	0		16	3	19
	SF	17	4	24	0	0		17	4	24

* Rose-Waaler titre $\geq 1:32$; ** Rose-Waaler titre $\leq 1:16$; *** for abbreviations see 'Materials and methods'; **** most of the patients studied were cases with ankylosing spinal involvements, active sacroileitis as well as suffered for peripheral joint manifestations.

Table 2. Acid unmasked 'hidden' RF in serum and synovial fluid samples and in their IC-enriched fractions of RA patients

		RF in 3% PEG precipitates after acidic dissociation	No RF in 3% PEG precipitates after acidic dissociation	3% PEG insoluble protein (μg/ml) mean ± SE*	3% PEG insoluble C4 (μg/ml) mean ± SE**
Serum	'Hidden' RF positive (n = 21)	17	4	262.4 ± 30.3	4.3 ± 0.9
	'Hidden' RF negative (n = 38)	10	28	229.3 ± 22.4	2.0 ± 0.4
			$p < 0.001$***	p = n.s.****	$p < 0.0025$
SF	'Hidden' RF positive (n = 23)	19	4	297.6 ± 41.9	3.5 ± 0.74
	'Hidden' RF negative (n = 17)	7	10	206.7 ± 49.7	1.35 ± 0.2
			$p < 0.005$	p = n.s.	$p < 0.001$

* Protein content of PEG fractions of 80 healthy persons (mean ± SE) = 105.3 ± 4.5 μg/ml; ** C4 content of PEG fractions of sera of 80 healthy persons (mean ± SE) = 0.34 ± 0.1 μg/ml; *** χ^2-probe (in sera) $\chi^2 = 16.2$, n = 59; in SFs $\chi^2 = 8.0$, n = 40); **** Student's t-test.

taken for the presence of complexes with special size or composition not precipitated by the PEG solution used in our experiments. Anyway, possibly because of the heterogeneity of ICs in samples, different approaches for measuring RF-ICs do not provide completely similar results.

At the same time, we found more than 3% PEG-insoluble protein and significantly higher amounts of complexed C4 in samples with positive 'hidden' RF than in negative ones. The level of complexed complement factors (e.g. C4) seems to provide rather sensitive and specific characterization of ICs[13]. As we found significantly more complexed C4 in ICs of 'hidden' RF positive samples than in those of the 'hidden' RF negative cases, the suggestion can be made that a close relation between the 'hidden' RF and IC-level might be considered.

In these experiments we used Rose-Waaler method at 37 °C, therefore unmasked 'hidden' RF antibodies detected are mainly IgM rheumatoid factors[14]. (Moreover, according to our previous unpublished observations, agglutinating activity of acid-unmasked RFs can be completely abolished by 0.1 M 2-mercapthoethanol.) As neutralization is done only immediately before titration of dissociated RF antibodies, separation from the dissociated ligands (IgG) in most of the cases is not necessary.

The mild acidic treatment to 'split' ICs in sera and SFs seems to offer a simple screening method to detect 'hidden' RFs.

At the same time, results presented here may raise the problems of conventional RF measurements. In certain cases the false-negative of false-low titres of RF antibodies may mean the presence of another pool of circulating RFs, masked in ICs. Therefore a more cautious evaluation of results of conventional laboratory RF tests is needed.

The parallel occurrence of 'hidden' RFs in sera or synovial fluids and in their IC-enriched fractions may suggest that the measurement of 'hidden' RFs in body fluids may permit a very simple estimation of the level of some IC-bound rheumatoid factors providing diagnostic possibility mainly for rheumatoid vasculitis.

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Local adjuvants: The influence of sodium dodecylbenzene sulphonate on immunization with aerosolized antigen[1]

R.J.F. Markham and B.N. Wilkie

Department of Veterinary Microbiology and Immunology, University of Guelph, Guelph (Ontario, Canada), 5 June 1978

Summary. Guinea-pig respiratory and serum antibody responses were enhanced following exposure to aerosols of bovine IgG_2 dissolved in solutions of sodium dodecylbenzene sulphate (SDBS). Enhanced response was seen in both primary and secondary immunization. Cell-mediated immune response (indirect macrophage migration influencing test) was not altered by SDBS. Results are discussed with a view to the possible utility of SDBS as adjuvant for prophylactic immunization.

Immunization by application of antigen directly onto mucosal surfaces is the most effective way of stimulating secretory immune responses[2]. Aerosol exposure to antigen can stimulate all levels of the respiratory immune response and in addition can elicit a good systemic response[3].

Despite the potential importance of aerosol immunization in disease prophylaxis, little attention has been given to the development of materials which could enhance the immune response to antigens given by the aerosol route, i.e. local adjuvants. The use of adjuvants systemically is well