Binding of isolated rheumatoid factors to histone proteins and basic polycations

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SUMMARY A fluorimetric immunoassay has been used to assess reactivity of rheumatoid factor (RF) with both histone proteins and other basic polycations (poly-L-lysine, poly-L-ornithine, and protamine) bound to an immobilised tyrosine-glutamic acid polyanionic copolymer. Isolated RF preparations can bind to histone proteins in this assay, notably to H3 and H4 histones, and this activity was always masked in the original whole seropositive sera. Binding of isolated RF was often noted also to the other large-molecular-weight basic polycations.

Several groups have reported that isolated rheumatoid factors (RF) may express reactivity for a cell nuclear determinant as well as for a determinant in the Fc region of IgG.¹⁻⁴ The reaction of RF with cell nuclei can often be masked in studies with whole seropositive sera because of the presence of a vast excess of serum IgG, and hence isolation of an RF fraction is usually important for its detection.^{2,3} However, this cross-reactive RF specificity is now considered to be of little immunopathological significance,⁵ though it may be of interest in clarifying concepts related to the characteristics of the variable region of RF antibody molecules and approaches towards understanding the genesis of RF production in patients with rheumatoid arthritis (RA).⁴

The cell nuclear determinants reactive with isolated RF are expressed in nuclei of normal cells from most species and are represented within isolated mononucleosome preparations.⁶ It has been concluded that these determinants are conformationally dependent, since they are destroyed when DNA and the core histone proteins are separated from their organised structure with nucleosomes.^{2,3,6} Thus, isolated histones in free solution are unreactive with RF preparations.^{2,3,6} The present study has utilised a sensitive fluorimetric assay for antihistone antibodies to assess RF reactivity with both histone proteins and other basic polycations bound to an immobilised tyrosine-glutamic acid (tyr-glu) copolymer. The spatial orientation of histone proteins bound to this

acidic copolymer may resemble that of these proteins bound to their natural polyanionic ligand, that is, DNA. It has been shown that isolated RF can bind to histone proteins, notably H3 and H4, held in this stereospecific form, and that RF may also bind to other polycations.

Materials and methods

RF preparations. RF preparations were isolated from seropositive RA sera of high titre by affinity chromatography using immobilised heat-aggregated IgG as described previously.^{2,5} One of the original whole sera (no. 2) gave weak homogeneous antinuclear antibody (ANA) staining on tissue section immunofluorescence (titre: 128), whereas the remainder of the whole sera showed no ANA activity. Isolated RF preparations were dialysed against phosphate-buffered isotonic saline, pH 7.2, concentrated to 1 mg/ml (1 g/l) protein and stored frozen in small aliquots with addition of 10% glycerol. All preparations showed RF reactivity with IgG in agglutination assays and also cross-reactivity with cell nuclei in tissue section immunofluorescence.² For use as a control an IgM κ preparation was isolated from the serum of a patient with Waldström's macroglobulinaemia as described elsewhere.⁷ No RF or ANA activity was detected in this patient's serum or isolated IgM κ paraprotein.

Antihistone antibody assay. Antihistone antibody activity was assessed by a fluorimetric immunoassay to be described in detail elsewhere (Hobbs and Lea, in preparation). Briefly, the tyr-glu copolymer (1:1 composition, molecular weight 66 000, from Miles

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Laboratories, Slough, UK) was absorbed by hydrophobic interaction on to polystyrene EIA cuvettes (Gilford Instruments, London, UK). This was performed by addition to each cuvette of 100 μ l polymer solution in 0.05 M borate buffer containing 50 mM NaCl, pH 8.2. After sealing with Parafilm M, the cuvettes were incubated at 20°C overnight and unbound polymer then removed by thorough washing with distilled water. Mixed histones (type II-AS, from Sigma Chemical Co., Poole, UK) derived by extraction of calf thymus, or individual histones purified from pig (for H1 and H3) or calf (for H2A, H2B, H4) thymuses* (a gift from Dr E. W. Johns, Institute of Cancer Research, Royal Cancer Hospital, London), were absorbed on to the polyanionic polymer surface by addition to each cuvette of 100 μ l of histone solution (100 mg/l) in the borate-NaCl buffer, pH 8.2, and incubated overnight as previously described. Unbound histones were then removed by thorough washing with the borate-NaCl buffer. A 1:20 dilution of test serum or 10 μ g/ml (10 mg/l) isolated RF preparation in the borate-NaCl buffer containing also 0.1% Tween 20 was added, incubated at 20°C overnight, and then washed thoroughly with the borate-NaCl-Tween buffer. Fluorescein isothiocyanate (FITC)-conjugated sheep antihuman immunoglobulin (Wellcome Reagents, Beckenham, UK) at 1:60 dilution in the borate-NaCl-Tween buffer containing also 5% normal sheep serum was added to each cuvette and incubated at 20°C for 6 hours before removal of unbound FITC-conjugate by thorough washing with the borate-NaCl-Tween buffer. Bound FITC-conjugate was then removed by addition of 750 μ l of 0.2% sodium dodecyl sulphate in 0.1 M NaOH and fluorescence of the resultant solution measured in a Locarte digital fluorimeter as described in detail by Lea and Ward.⁹ The fluorimeter was calibrated such that a 1:12 000 dilution of the FITC-conjugate alone gave a reading of 1.00.

Basic polycations. Poly-L-lysines (PLL) of mean molecular weights (MWs) 350 000, 13 000, and 3000, poly-L- α -ornithine (mean MW 40 000) and salmon sperm protamine (grade IV) were obtained from Sigma Chemical Co., Poole, UK. Reactivity with RF or non-RF IgM preparations was assessed by fluorimetric immunoassay essentially as described above but with these polycations absorbed directly on to plain polystyrene EIA cuvettes after overnight incubation at 20°C at a concentration of 100 mg/l in the borate-NaCl buffer, pH 8.2.

Results

The binding activity to histone proteins in the fluorimetric assay for paired samples of whole seropositive RA sera and corresponding RF preparations is given in Table 1. These values for sera and RF preparations are compared with those of a whole normal human serum (lacking RF and ANA activity) and an isolated non-RF IgM κ protein. Binding to the polyanionic tyr-glu copolymer alone in all cases was very low. Only one of the RA sera (no. 2) showed significant antihistone antibody activity. This reactivity was not strong and was manifest against all individual histones and a mixed histone preparation, although reactivity with H1 and H2A was slightly more pronounced (Table 1). In contrast, 2 of the 3 isolated RF preparations (nos. 1 and 3) showed significant reactivity compared with non-RF IgM, and this reactivity was most pronounced against H3 and H4 histone proteins (Table 1). When RF preparation no. 3 was added to a normal human serum (final concentration 20 μ g/ml (20 mg/l) in a 1:20 dilution of serum), the fluorescence value for reactivity against each of the histone preparations was reduced in every case to less than 20%. With the use of FITC-labelled mixed histones the amount of histone bound to the immobilised polyanionic tyr-glu copolymer was determined to be 3.0 times greater

Table 1 Histone-binding activity in 3 paired seropositive RA sera and isolated RF preparations

Histone preparation	Fluorescence value							
	Whole sera				RF preparations:			
	No. 1	No. 2	No. 3	NHS	No. 1	No. 2	No. 3	Non-RF IgM
Mixed histones	0.16	0.41	0.13	0.06	1.31	0.73	1.76	0.63
H1	0.17	0.53	0.09	0.06	0.45	0.12	0.73	0.33
H2A	0.17	0.59	0.10	0.04	0.83	0.25	1.20	0.50
H2B	0.14	0.38	0.14	0.04	0.95	0.44	1.22	0.48
H3	0.17	0.36	0.15	0.07	1.42	0.82	1.87	0.58
H4	0.14	0.34	0.15	0.12	1.40	0.78	1.80	0.55
No added histone	0.03	0.05	0.05	0.01	0.03	0.04	0.06	0.02

NHS=whole normal human serum.

Table 2 Basic polycation-binding activity of isolated RF preparations

Basic polycation	Mean MW	Fluorescence value					
		RF preparations					
		No. 1	No. 2	No. 3	No. 4	Non-RF IgM	
PLL	350 000	1.65	0.43	2.20	0.86	0.39	
PLL	13 000	0.97	0.34	0.58	0.17	0.21	
PLL	3 000	0.88	0.15	NT	NT	0.01	
Polyornithine	40 000	0.99	0.16	1.90	0.87	0.31	
Protamine	5 000	3.35	0.21	0.13	0.06	0.06	

PLL=poly-L-lysines. NT=not tested.

than that bound to the plain polystyrene cuvettes, whereas RF reactivity with mixed histones was increased 6–10 fold.

The binding activity of isolated RF preparations for different basic polycations in the fluorimetric assay is given in Table 2. Three of 4 RF preparations bound significantly to high molecular weight PLL, and this activity decreased markedly for PLL polymers of lower molecular weights (Table 2). Furthermore, 3 RF preparations showed significant binding to polyornithine, and one preparation also bound strongly to protamine (Table 2).

Discussion

It has been established that both DNA and histones might be involved in the expression of the cell nuclear determinants reactive with isolated RF^{2,3,6} and presumed that these determinants may be expressed on histones only when orientated in a particular conformation on DNA.^{3,4} The reactivity of isolated RF preparations with individual and mixed histones extracted from nuclei has been investigated in a fluorimetric immunoassay with a solid-phase coated with an polyanionic tyr-glu copolymer. It has been shown¹⁰ that polyanions produced from glutamic and acid or aspartic acid mimic assembly proteins¹¹ and can organise core histone proteins into octamers similar to those found in nucleosomes. The structure of histone mixtures bound to the polyanionic tyr-glu copolymer may resemble that of these proteins bound to DNA.

RF preparations can bind to histone proteins in this assay, notably to H3 and H4 histones, and this activity was always masked in the original whose seropositive sera used in this study. However, it is of note that whole sera of RA patients with systemic vasculitis often show greatest antihistone antibody activity against both H2A and H3 histones, unlike, for example, the sera of systemic lupus erythematosus

patients (Hobbs and Lea, in preparation). In contrast to the present report a previous study⁹ has suggested that RF cross-reactivity with histone proteins may be particularly directed to the H2A and H2B histones. This was assessed by experiments in which solutions containing purified histone fractions were added to cell nuclei that had been stripped by mild acid treatment. The constitution of structures produced by this technique has not been described, although it is known that reconstitution of nucleosomes with soluble DNA usually requires the presence of an assembly protein,¹¹ whereas DNA precipitates histones in solution.

Isolated RF preparations have been shown to bind to other high-molecular-weight polycations such as poly-L-lysine, poly-L-ornithine, and protamines immobilised directly on plain polystyrene. Thus the binding of RF to nucleosomes may be at locations primarily determined by the availability of free basic groups. Indeed the reactivity of isolated RF preparations with polycations, including histone proteins, may at present best not be described as immunological cross-reactions with different antigenic groupings, which implies multiple specificities contributing to the antigen-binding site in the hypervariable regions of RF immunoglobulin molecules. Rather it would appear that there may be at least one unusual structure within the variable region of some RF molecules, but not necessarily integral within the finite antigen combining site of the RF molecules, that promotes apparent reactivity of this protein with a variety of basic polycations. However, other factors than ionic interactions may contribute to such RF binding, since the binding of isolated RF with cell nuclei is optimal at more alkaline pH,⁵ when there would be less ionisation of basic groupings, and also it has been reported that isolated RF may bind to nonbasic groupings such as the di- and trinitrophenyl haptenic groups.¹²

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