

Common IgA and IgM rheumatoid factor idiotypes in autoimmune diseases

A E GHARAVI,¹ B M PATEL,¹ G R V HUGHES,¹ AND K B ELKON²

From the ¹Rheumatology Unit, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 0HS, and the ²Hospital for Special Surgery, 535 East 70th Street, New York, NY 10021.

SUMMARY Anti-idiotypic antibodies to four monoclonal IgM, one polyclonal IgM, and one polyclonal IgA rheumatoid factors were produced in rabbits and tested for cross reactivity with rheumatoid factors from nine patients with essential mixed cryoglobulinaemia, three patients with rheumatoid arthritis, four patients with systemic sicca syndrome, and one patient with systemic lupus erythematosus. Variable (3/16 to 10/16) cross reactivity for each anti-idiotypic antibody was observed which was not restricted by disease category, clonality, or isotype of the rheumatoid factor. These findings suggest that rheumatoid factor genes are highly conserved from the germ-line antibody repertoire.

Key words: anti-idiotypic, rheumatoid arthritis, systemic sicca syndrome, essential mixed cryoglobulinaemia.

Rheumatoid factors (RF) are low affinity antibodies with specificity for IgG.¹ These autoantibodies circulate at low levels in normal sera,² at high levels in many infectious and autoimmune diseases,³ and are readily induced by exposing lymphocytes to mitogens *in vitro*.⁴ Although IgM RF have been most extensively studied, IgA^{5,6} and IgG⁷ rheumatoid factors have also been characterised. It has been suggested that RF are part of the immune network^{8,9} and may serve to facilitate clearance of antigen-antibody complexes.¹⁰ In order to investigate the structural relationship between RF of different clonality, different isotype, and in different diseases, we made anti-idiotypic antibodies to purified RF from several sources. These anti-idiotypic antibodies showed extensive cross reactivity within and between disease categories and even between RF of different isotype.

Materials and methods

PREPARATION OF RHEUMATOID FACTORS (RF)
Sera from 17 patients known to have high rheumatoid factor activity were selected for our study. The diagnoses were: essential mixed cryoglobulinaemia (EMC, nine patients), rheumatoid arthritis (RA, three patients), systemic sicca syndrome (SSS, four

patients), and systemic lupus erythematosus (SLE, one patient). The IgM fraction of each serum was prepared by euglobulin precipitation and chromatography on a Sepharose 6B column equilibrated with 0.1 M acetate buffer, pH 4.0. The IgM peak was dialysed against phosphate-buffered saline (PBS, 0.15 M sodium chloride buffered with 0.01 M phosphate, pH 7.2) and concentrated to approximately 1-2 g/l. Each preparation was tested for contaminating IgA or IgG by Ouchterlony immunodiffusion with commercial antisera (Seward Laboratory, UK). All IgM RF used as immunogens and two IgA RF were affinity purified from columns of heat-aggregated human IgG coupled to Sepharose 4B as described previously.¹¹ Contaminating immunoglobulins were removed by passage through F(ab')₂anti-μ or F(ab')₂anti-α affinity columns.¹¹ RF were characterised as monoclonal or polyclonal on the basis of their reactivity with anti-kappa and anti-lambda antisera (Seward Laboratory, UK), and by immunoelectrophoresis with commercial anti-whole human antisera (Immunophor, Vienna).

PREPARATION OF ANTI-IDIOTYPES

Rabbit antisera to five different IgM RF and one IgA RF were produced by immunising New Zealand rabbits with the affinity-purified RF. Immunisation was carried out with 500 μg protein emulsified in Freund's adjuvant given at multiple sites both subcutaneously and intramuscularly on at least four occasions at three-week intervals. Each antiserum

Accepted for publication 21 September 1984.

Correspondence to A E Gharavi MD, Rheumatology Unit, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 0HS.

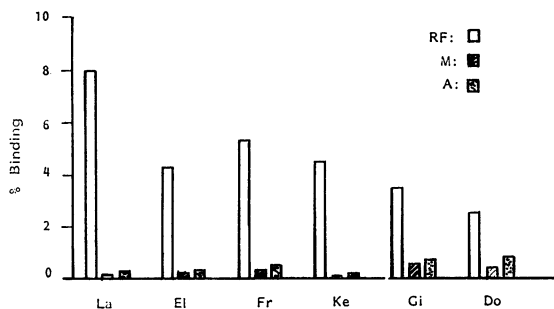


Fig. 1 The relative binding specificities of anti-idiotypes against monoclonal IgM rheumatoid factors (RF) (La, El, Fr, Ke), polyclonal IgM RF (Gi), and polyclonal IgA RF (Do). RF=autologous RF, M=non-RF IgM, A=non-RF IgA.

was extensively absorbed with glutaraldehyde insolubilised¹² normal human serum as well as partially purified non-rheumatoid factor paraproteins of the same isotype as the immunogen. F(ab')₂ fractions of IgG were prepared by ammonium sulphate precipitation, diethylaminoethyl (DEAE) chromatography, and pepsin digestion as described previously.¹¹ F(ab')₂ fractions were separated from undigested IgG and Fc' by Sephadex G100 chromatography and were concentrated to at least 1 g/l in PBS. The specificity of each anti-idiotype reagent was tested by a direct binding assay, whereby equal amounts of immunogen or non-RF immunoglobulins were used to coat microtitre plates, and the binding of the ¹²⁵I-labelled¹³ anti-idiotype measured after 8 hours' incubation at room temperature (Fig. 1). The F(ab')₂ anti-idiotype is hereafter referred to as anti-Id.

RHEUMATOID FACTOR BINDING AND INHIBITION ASSAYS

The microplate RF radioimmunoassay with human IgG as antigen and ¹²⁵I-F(ab')₂ anti-μ or anti-α to detect RF isotypes has been described previously.^{5,6} Each RF preparation was serially diluted in PBS and bovine serum albumin (BSA) (1% w/v BSA in PBS) to find the concentration at which binding was reduced to 50% of maximum (50% activity). The inhibition studies were performed by preincubating RF at 50% activity with increasing concentrations of anti-Id in PBS-BSA for 60 minutes at 37°C and at 4°C overnight prior to the RF radioimmunoassay (Fig. 2). To control for any effect of dilution, the percentage inhibition of RF binding due to the anti-Id was calculated after subtraction of a control sample diluted with PBS-BSA as follows:

$$\% \text{ inhibition} = \frac{\text{binding control} - \text{binding anti-Id}}{\text{binding control}} \times 100$$

Inhibition of RF binding was also calculated after addition of pooled F(ab')₂ fraction prepared from IgG of non-immunised rabbits. All studies were performed in duplicate on at least two separate occasions. Since the intra-assay variation was approximately 5%, inhibition ≥10% was regarded as significant.

Results

CHARACTERISATION OF ANTI-IDIOTYPE

Each of the isolated RF preparations was shown to contain only one immunoglobulin isotype (either IgM or IgA) by Ouchterlony immunodiffusion. The relative specificities of the anti-Id raised against the 5 IgM RF (La, El, Fr, Ke are monoclonal and Gi is a polyclonal IgM RF) and one IgA RF (Do is a polyclonal IgA RF) are shown in Fig. 1. The anti-Id directed towards IgM RF therefore showed a specificity (binding of anti-Id to immunogen/binding of anti-Id to non-RF IgM) of greater than 20 (anti-Fr, Gi, El) or greater than 100 (anti-La, Ke). Despite extensive absorptions of different anti-Do sera, the relative specificity of the anti-Id for IgA RF was only five.

CROSS-REACTIVE IDIOTYPES (CRI)

The percentage inhibition of binding of IgM and IgA RF by each anti-Id is shown in Table 1. Two of the anti-Id (anti-La and anti-El), showed little cross reactivity with other IgM RF, whereas the remaining three anti-Id to IgM RF displayed extensive cross reactivity, reacting with six to eight of the 13 non-immunogen IgM RF. The anti-Id raised against IgA RF Do, also showed extensive cross reactivity inhibiting eight out of 14 IgM RF. This anti-Id also inhibited the three IgA RF. Surprisingly the two anti-Id (anti-La and anti-El) with the least cross

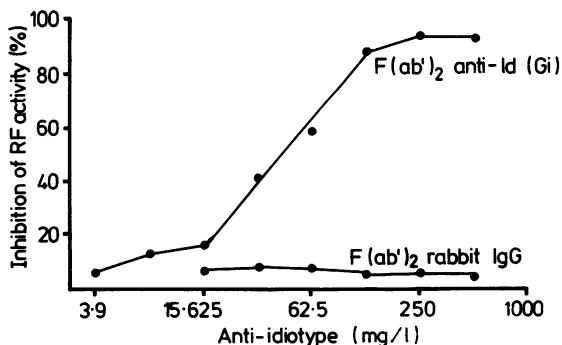


Fig. 2 Inhibition of (Gi) IgM rheumatoid factor (RF) activity by increasing concentrations of autologous anti-idiotype or F(ab')₂ fraction of normal rabbit IgG.

Table 1 Percentage inhibition of rheumatoid factor activity by various anti-idiotypic antibodies

Patient	Diagnosis*	RF†	Anti-La	Anti-El	Anti-Fr	Anti-Ke	Anti-Gi	Anti-Do
1	EMC	mM _x	79	—‡	—	—	—	31
2	EMC	mM _x	22	79	12	—	—	—
3	EMC	mM _x	—	—	97	29	26	—
4	EMC	mM _x	—	—	22	80	27	—
5	EMC	mM _x	—	—	—	—	—	—
6	EMC	mM _x	—	—	30	37	43	12
7	SSS	pM	—	52	—	—	16	47
8	EMC	pM	—	—	39	10	36	12
9	EMC	pM	—	—	—	28	34	15
10	EMC	pM	—	—	—	10	—	—
11	SLE	pM	—	—	12	17	10	17
12	RA	pM	—	—	11	—	86	—
13	RA	pM	—	—	—	12	13	58
14	RA	pM	—	—	—	—	—	45
15	SSS	pA	21	15	25	—	—	73
16	SSS	pA	18	13	—	—	—	11
17	SSS	pA	—	—	—	—	—	33

*EMC=essential mixed cryoglobulinaemia, SLE=systemic lupus erythematosus, RA=rheumatoid arthritis, SSS=systemic sicca syndrome.

†RF=rheumatoid factor; characterised as monoclonal(m), polyclonal(p), IgM(M), or IgA(A).

‡—=no inhibition.

reactivity for IgM RF inhibited two out of three IgA RF. For all of the above the degree of inhibition of binding varied considerably but was greatest for the immunising RF (Fig. 2). Cross-reactive idiotypes were therefore demonstrated between monoclonal and polyclonal IgM RF, between RF of different isotypes, and between RF generated in a variety of autoimmune diseases.

Discussion

Common antigenic determinants on IgM RF from patients with mixed cryoglobulinaemia were described in 1971.¹⁴ Cross-reactive (shared) idiotypes were subsequently demonstrated on monoclonal¹⁵ and polyclonal¹⁶ IgM RF. In the current study we raised rabbit anti-RF idiotypic antibodies against both mono- and polyclonal RF and against IgM and IgA RF. Although all four monoclonal IgM RF used as immunogens to raise anti-idiotypic antisera contained kappa light chains, considerable variation in cross reactivity with other IgM RF was observed (14–57%, Table 1). These findings indicate that a number of shared idiotypes exist on both mono- and polyclonal IgM RF, and that the anti-Id antisera are not merely recognising common structural determinants on the KIIIb light chain associated with monoclonal IgM RF.¹⁷ An anti-Id raised against the polyclonal IgM RF (Gi) cross reacted with three out of six monoclonal IgM RF and six out of eight polyclonal IgM RF. Whether this high frequency of cross reactivity is due to the presence of several different idiotypic-anti-idiotypic interactions with

polyclonal reagents, or the presence of a dominant idiotypic similar to the Ke IgM RF used in this study, is uncertain. Forre *et al.* were unable to identify distinct cross idiotypic groups among polyclonal RF in rheumatoid arthritis.¹⁶

It is not known whether the IgA RF found in the serum of patients with rheumatoid arthritis and the sicca syndrome are derived from successive class switching of IgM-RF-producing plasma cells or whether these polymeric antibodies arise from independent clones of cells.^{5,6} The presence of cross-reactive idiotypes among IgA and IgM RF provides evidence in favour of the former hypothesis, but does not prove it. Whereas the broad cross reactivity noted with the anti-Do IgA RF may be, in part, due to its lower specificity, the reactivity of three highly specific antimono-clonal IgM reagents with IgA RF cannot be explained by technical factors. In addition, cross inhibition of polyclonal IgA RF by a mouse monoclonal anti-idiotypic raised against a monoclonal IgM RF has recently been described.¹⁸ Together, these findings strongly suggest that shared idiotypes may occur in antibodies of a different isotype.

In this study cross-reactive idiotypes were found to be shared in RF from unrelated patients with four different diseases. It seems likely, therefore, that rheumatoid factor genes are highly conserved in the germ-line antibody repertoire and are readily activated in autoimmune diseases.

This work was supported by the Arthritis and Rheumatism Council, UK, and Grants AM 14627 and AM 32845 from the National Institutes of Health, Bethesda, USA.

References

- 1 Franklin E C, Holman H R, Muller-Eberhard H J, Kunkel H G. An unusual protein component of high molecular weight in the serum of certain patients with rheumatoid arthritis. *J Exp Med* 1957; **105**: 425-38.
- 2 Muller-Eberhard H J, Kunkel H G. Isolation of a thermolabile serum protein which precipitates γ -globulin aggregates and participates in immune haemolysis. *Proc Soc Exp Biol Med* 1961; **106**: 291-5.
- 3 Carson D A, Pasquali J L, Tsoukas C D, et al. Physiology and pathology of rheumatoid factors. *Springer Semin Immunopathol* 1981; **4**: 161-79.
- 4 Dresser D W. Most IgM-producing cells in the mouse secrete autoantibodies (rheumatoid factor). *Nature* 1978; **274**: 480-3.
- 5 Elkon K B, Delacroix D L, Gharavi A E, Vacrman J P, Hughes G R V. Immunoglobulin A and polymeric IgA rheumatoid factors in systemic sicca syndrome: partial characterisation. *J Immunol* 1982; **129**: 576-81.
- 6 Elkon K B, Gharavi A E, Patel B M, Hughes G R V, Frankel A. IgA and IgM rheumatoid factors in serum, saliva and other secretions: relationship to immunoglobulin ratios in systemic sicca syndrome and rheumatoid arthritis. *Clin Exp Immunol* 1983; **52**: 75-84.
- 7 Pope R M, Teller D C, Mannik M. The molecular basis of self-association of antibodies to IgG (rheumatoid factors) in rheumatoid arthritis. *Proc Natl Acad Sci USA* 1974; **71**: 517-21.
- 8 Geltner D, Franklin E C, Frangione B. Anti-idiotypic activity in the IgM fractions of mixed cryoglobulins. *J Immunol* 1980; **125**: 1530-5.
- 9 Kunkel H G, Posnett D N, Pernis B. The anti-immunoglobulins and their idiotypes: are they part of the immune network? *NY Acad Sci* 1983; **418**: 324-9.
- 10 Abruzzo J L, Christian C L. The induction of a rheumatoid factor-like substance in rabbits. *J Exp Med* 1961; **114**: 791-806.
- 11 Elkon K B, Caciro F, Gharavi A E, Patel B M, Ferjencik P P, Hughes G R V. Radioimmunoassay profile of antiglobulins in connective tissue diseases: elevated level of IgA antiglobulin in systemic sicca syndrome. *Clin Exp Immunol* 1981; **46**: 547-56.
- 12 Avrameas S, Ternynck T. The cross-linking of proteins with glutaraldehyde and its use for the preparation of immunoadsorbents. *Immunochemistry* 1969; **6**: 53-66.
- 13 McConahey P J, Dixon F J. A method of trace iodination of proteins for immunologic studies. *Int Arch Allergy Appl Immunol* 1966; **29**: 185.
- 14 Franklin E C, Frangione B. Common structural and antigenic properties of human γ M anti- γ globulins. *J Immunol* 1971; **107**: 1527-34.
- 15 Kunkel H G, Agnello V, Joslin F G, Winchester R J, Capra J D. Cross-idiotypic specificity among monoclonal proteins with anti-globulin activity. *J Exp Med* 1973; **137**: 331-42.
- 16 Forre O, Doblough J H, Michaelsen T E, Natvig J B. Evidence of similar idiotypic determinants on the different rheumatoid factor populations. *Scand J Immunol* 1979; **9**: 281-9.
- 17 Ledford D K, Goni F, Pizzolato M, Franklin E C, Soloman A, Frangione B. Preferential association of KIIIB light chains with monoclonal human IgM_k autoantibodies. *J Immunol* 1983; **131**: 1322-5.
- 18 Carson D A, Fong S. A common idiotope on human rheumatoid factors identified by a hybridoma antibody. *Mol Immunol* 1983; **20**: 1081-7.