

# EVIDENCE FOR A SUBSET OF RHEUMATOID FACTORS THAT CROSS-REACT WITH DNA-HISTONE AND HAVE A DISTINCT CROSS-IDIOTYPE\*

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Rheumatoid factors (RF)<sup>1</sup> have been shown to have considerable heterogeneity of specificities for antigenic determinants on the IgG molecule (1, 2). Among monoclonal RF, heterogeneity has been also demonstrated by the presence of different cross-idiotype antigens that are thought to relate to differences in antibody-combining sites (3). In recent years, reports of RF that cross-react with non-IgG antigens has suggested that still greater heterogeneity exists among RF than previously thought (4, 5). In preliminary publications (6, 7) we have reported that a monoclonal RF (mRF) isolated from the serum of a patient with Waldenström's macroglobulinemia cross-reacted with DNA-nucleoprotein and that similar cross-reactive polyclonal RF appear to occur in pathologic fluids in a variety of human diseases. We now provide direct evidence that certain RF react with an antigen that appears to be present on both IgG and DNA-nucleoprotein. In addition, evidence is presented to support the hypothesis that these RF comprised a subset which is determined not only by their cross-reactivity with DNA-nucleoprotein but by the presence of a cross-idiotype antigen distinct from those previously identified among mRF (3).

## Materials and Methods

*Preparation of Reagents.* An IgM  $\kappa$ -RF (Bla mRF) was isolated by IgG-Sepharose (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N. J.) affinity chromatography (8). The preparation was 98.7% IgM, 0.4% IgG, and 0.9% IgA. C1q was not detectable. Polyclonal RF (Orl) was isolated in a similar fashion. Other mRF were isolated from cryoglobulins as previously described (9). F(ab')<sub>2</sub> and Fab fragments of Bla mRF were prepared by 2.0% pepsin (wt:wt) digestion at 37°C for 4 h at pH 4.0, and were isolated by G150-Sephadex (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc.) chromatography. The Fab fragment was also prepared by trypsin digestion at 63°C. (10) Nuclei and DNA-nucleoprotein were isolated from calf thymus (11). Soluble DNA-nucleoprotein (sNP) was prepared by ultrasonic disintegration (Sonifier, Heat Systems-Ultrasonics, Inc., Plainview, N. Y.). Concentrations of DNA-nucleoprotein were determined by the diphenylamine assay for DNA and micro-biuret assay for

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<sup>1</sup> *Abbreviations used in this paper:* ANF, antinuclear fluorescence; Bla mRF, an IgM rheumatoid factor; BSA, bovine serum albumin; K<sub>0</sub>, association constant(s); LE, lupus erythematosus; McD, a monoclonal rheumatoid factor without DNA nucleoprotein reactivity; L. serum, serum from a patient with systemic lupus erythematosus known to have an anti-nucleoprotein antibody without reactivity to aggregated IgG; MCTD, mixed connective-tissue disease; mRF, monoclonal rheumatoid factor(s); Orl, a polyclonal rheumatoid factor; PBS, phosphate-buffered saline; RA, rheumatoid arthritis; RF, rheumatoid factor(s); RO, rheumatoid overlap; SLE, systemic lupus erythematosus; sNP, soluble-DNA nucleoprotein.

protein. Mono-, oligo-, and polynucleosomes were prepared from isolated calf thymus nuclei as described (12). The size of the mononucleosomes was confirmed as 11S by analytical ultracentrifugation in a Beckman model E ultracentrifuge (Beckman Instruments, Inc., Spinco, Div., Palo Alto, Calif.) Purified aggregated human IgG was prepared as previously described (8). Solid-phase preparations of RNAase, DNAase, and pronase were prepared by conjugation to Sepharose using the method of Bing (13).

*Preparation of Antisera.* Antisera with cross-idiotypic specificity for two groups of monoclonal anti- $\gamma$ -globulins: the Wa group, anti-Ma (an mRF without DNA-nucleoprotein reactivity [anti-McD in this study]); and the Po group, anti-Lay (an mRF without DNA-nucleoprotein reactivity) were raised and characterized in a previous study (3). Antisera to Bla mRF, Lay mRF, and Orl RF were raised by a minimum of 8 weekly immunizations of rabbits or goats with 0.5 mg of the respective protein in complete Freund's adjuvant. Antisera were absorbed with 0.2 ml of normal serum, 2.0 mg of Cohn Fraction II, and 2.0 mg each of a  $\kappa$ - and  $\lambda$ -IgM protein without anti- $\gamma$ -globulin activity per ml of antiserum. F(ab')<sub>2</sub> fragments of IgG isolates were prepared by pepsin digestion at 37°C for 18 h at pH 4.0, and isolated on G150-Sephadex. F(ab')<sub>2</sub> preparations were further absorbed by passage over solid-phase  $\kappa$ - and  $\lambda$ -IgM proteins without anti- $\gamma$ -globulin activity.

*Immunofluorescent Techniques.* Fluorescein-conjugated reagents and direct and indirect techniques for demonstrating antinuclear antibody were performed as previously described (14). To screen for the presence of cross-reactive RF in sera, tests employing blocking of the antinuclear fluorescence reaction (ANF) were performed. Equal volumes of a dilution of the test serum which gave 2<sup>+</sup> fluorescence, and 1 mg/ml solutions of aggregated IgG or sNP were incubated for 1 h at 20°C, centrifuged, and the ANF activity of the supernate compared to that of controls blocked with bovine serum albumin (BSA). Complete blocking by both aggregated IgG and sNP was considered a positive test for cross-reactive RF. Characterization of Bla and Orl ANF reactions on acid-eluted and histone-reconstitution tissues was performed as previously described (15, 16).

*Assays.* Reagents were labeled with I<sup>125</sup> using the lactoperoxidase-catalysed reaction previously described (8). The sp act obtained were: mRF: 0.1–0.25  $\mu$ Ci/ $\mu$ g, Bla mRF F(ab')<sub>2</sub>: 0.03  $\mu$ Ci/ $\mu$ g, sNP: 0.05  $\mu$ Ci/ $\mu$ g, polynucleosomes: 0.02  $\mu$ Ci/ $\mu$ g, and human IgG: 0.03,  $\mu$ Ci/ $\mu$ g. The one-step competitive inhibition assay of <sup>125</sup>I-Bla mRF binding to IgG-Sepharose has been described in detail (8). Studies with other radiolabeled mRF were performed in the same manner. Briefly, 100  $\mu$ l buffer or inhibitor and 100  $\mu$ l <sup>125</sup>I-mRF (~0.2  $\mu$ g) were added to 250  $\mu$ l of 1.25% suspension of IgG-Sepharose. 550  $\mu$ l of phosphate-buffered saline (PBS) was added and the tubes rotated overnight at 4°C. Tubes were spun, washed three times with PBS, the pellet counted in a gamma counter (Beckman Instruments, Fullerton, Calif.), and the percent inhibition calculated.

DNA-nucleoprotein coupled to Sepharose produced a ligand which was unable to bind Bla mRF; therefore, a double antibody technique was used for measuring <sup>125</sup>I-sNP or <sup>125</sup>I-polynucleosomes binding. 100  $\mu$ l mRF or serum specimen was incubated at 20°C for 10 min with either 100  $\mu$ l buffer or inhibitor. 100  $\mu$ l (1 $\mu$ g) <sup>125</sup>I-sNP or <sup>125</sup>I-polynucleosomes and then 700  $\mu$ l buffer were added. Tubes were rocked at 20°C for 1 h. 100  $\mu$ l of a goat anti-human Fc $\mu$  was added. After 1 h at 20°C, the precipitate was collected, washed three times with PBS, counted, and the percent binding or the percent inhibition calculated.

The assay that employed <sup>125</sup>I-sNP was used for screening sera for the presence of cross-reactive RF. 2  $\mu$ g of Bla mRF gave 50% binding which was completely inhibited by 10  $\mu$ g aggregated IgG or 10  $\mu$ g sNP. Equivalent binding by IgM anti-DNA nucleoprotein antibody isolated from serum from a patient with systemic lupus erythematosus (SLE) known to have an anti-nucleoprotein antibody without reactivity with aggregated IgG (L. serum) was inhibited by 10  $\mu$ g sNP but not 10  $\mu$ g aggregated IgG. The upper limit of binding for 55 normal sera was 4.5% (mean + 2 SD). Sera with greater amounts of binding were further tested to determine if aggregated IgG (10  $\mu$ g) blocked the binding. Greater than 50% blocking was considered a positive test for cross-reactive RF.

Hemagglutination inhibition experiments for demonstrating cross-idiotypic specificity of antisera were performed as previously described (3).

### Results

*Characterization of Bla mRF Reactivity with Aggregated IgG and DNA-Nucleoprotein.* The cross-reactivity of Bla mRF with a nuclear antigen was first demonstrated with the ANF reaction (Fig. 1 A). The ANF reaction could be blocked with aggregated IgG at 1 mg/ml (Fig. 1 B). In contrast the ANF reaction of the serum L. could not be blocked by aggregated IgG in concentration up to 20 mg/ml.

The cross-reaction could also be demonstrated in gel diffusion as shown in Fig. 2 A. Confirmation that Bla mRF reacted with an antigen involving DNA and protein was obtained by the type of enzymatic digestion experiments illustrated in Fig. 2 B. The possibility that the reactions were a result of contaminants, either DNA-nucleoprotein in the aggregated IgG or bovine IgG aggregates in DNA-nucleoprotein, was also ruled out in this experiment. In addition, it is known that Bla mRF does not precipitate

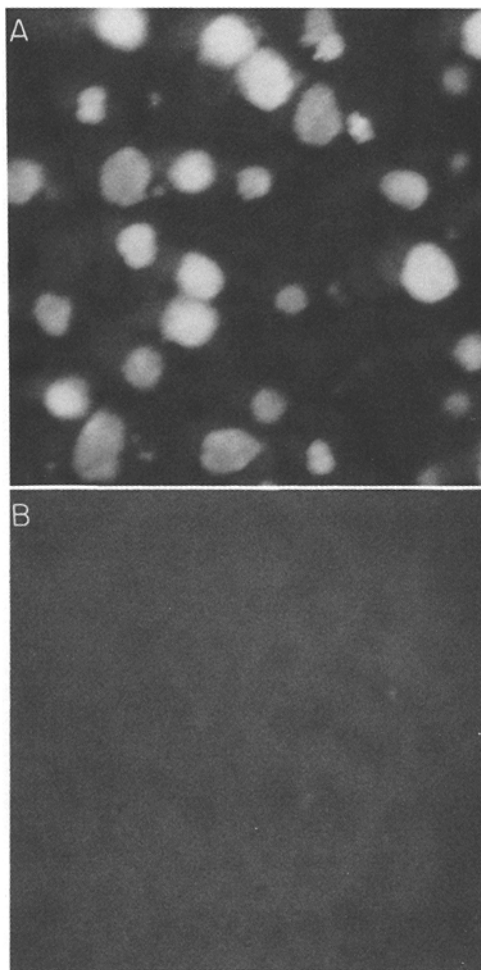


FIG. 1. (A) 4- $\mu$ m mouse liver section stained with 0.06 mg/ml fluorescein-labeled Bla mRF. Staining was obtained with concentrations as low as 0.002 mg/ml.  $\times$  1,350. (B) Fluorescence shown in (A) was blocked by incubating 1 mg/ml aggregated IgG with the fluorescein-labeled Bla mRF. Blocking was not obtained with 10 mg/ml BSA, 1 mg/ml DNA, or 1 mg/ml RNA.  $\times$  1,350.

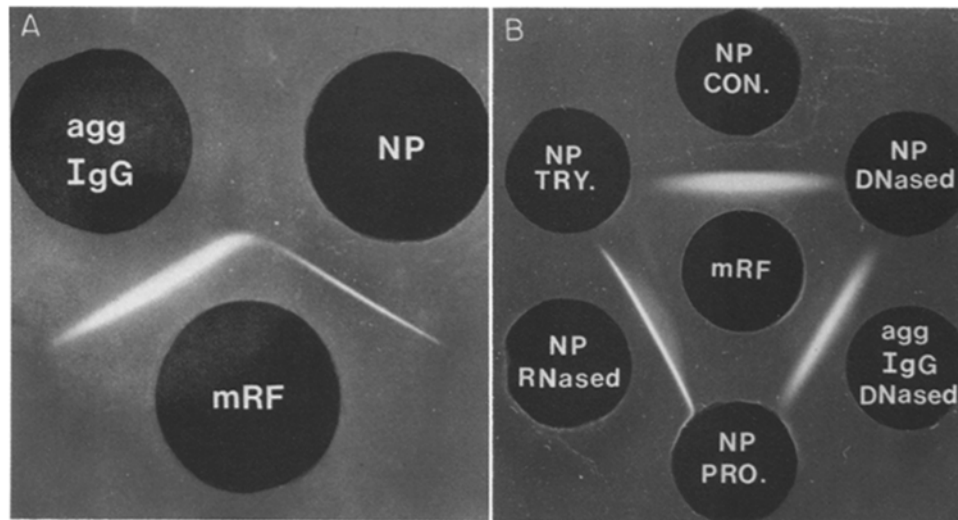


FIG. 2. 0.6% agarose gel diffusion plates. Precipitation of Bla mRF 1 mg/ml with aggregated 10 mg/ml IgG (agg IgG) and 3 mg/ml sNP (NP) (A). aggregated IgG treated with DNase (agg IgG DNased) or sNP treated with DNase (NP DNased), RNase (NP RNased), trypsin (NP TRY.), pronase (NP PRO.), or no enzyme (NP CON.) (B).

with bovine IgG (8); and in contrast to aggregated IgG where the antigenicity is increased by heating at 63°C, the reactivity of similarly heated DNA-nucleoprotein with Bla mRF is decreased. The remote possibility that trace amounts of C1q in the Bla mRF preparation were responsible for the reactions observed was eliminated by heating the mRF at 56° for 30 min before use.

Cross-reactions of the type shown by Bla mRF could not be found among heterologous antisera to IgG, sera from patients with SLE that gave strong precipitin reactions with DNA-nucleoprotein, or among eleven other IgM  $\kappa$ -mRF that gave strong precipitin reactions with aggregated IgG.

A sensitive competitive inhibition radioimmunoassay had been previously developed with Bla mRF (8). The binding of Bla mRF to IgG-Sepharose in this assay could be maximally inhibited by both aggregated IgG and DNA-nucleoprotein, whereas control proteins and nucleic acids gave no significant inhibition. Similar results could be demonstrated using a F(ab')<sub>2</sub> fragment of Bla mRF as shown in Fig. 3. The same effects of enzyme treatment shown in the precipitin experiments could also be demonstrated in the radioimmunoassay system.

Further evidence that the cross-reaction was via the antigen-combining site of the mRF and not through nonspecific interaction of IgM with aggregated IgG and DNA-nucleoprotein was obtained using an anti-idiotypic sera to Bla mRF. The F(ab')<sub>2</sub> preparation of this antiserum blocked the binding of Bla mRF to both aggregated IgG and DNA-nucleoprotein over a range of dilution as shown in Fig. 4. The binding of control antibodies, McD and antibodies from L. serum were not significantly inhibited by the anti-idiotypic F(ab')<sub>2</sub>.

*Studies of IgG and DNA-Nucleoprotein Antigens Reactive with Bla mRF.* Previous studies (8) have shown that Bla mRF reacts with aggregates of all subclasses of human IgG and rabbit IgG but not mouse, bovine, or sheep IgG. The Fc, but not the F(ab')<sub>2</sub>,

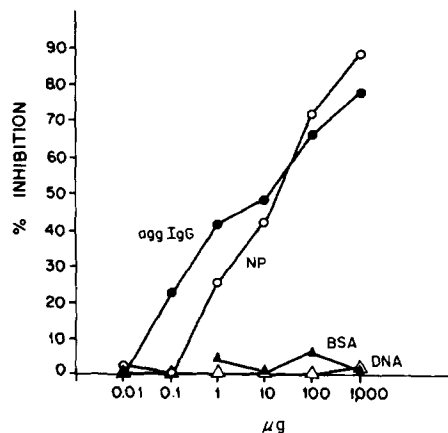


FIG. 3. Inhibition of  $0.1 \mu\text{g } ^{125}\text{I-Bla mRF F(ab')}_2$  binding to IgG-Sepharose by increasing amounts of aggregated IgG (●), NP (○), BSA (▲), and DNA (△).

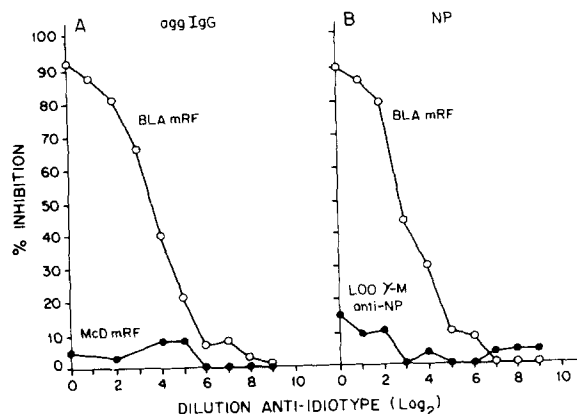


FIG. 4. Inhibition of antigen binding of Bla mRF by dilutions of 1.5 mg/ml  $\text{F(ab')}_2$  preparation of an anti-idiotype sera to Bla mRF. (A) Inhibition of  $0.1 \mu\text{g } ^{125}\text{I-Bla mRF}$  (○) and  $0.1 \mu\text{g } ^{125}\text{I-McD mRF}$  (●) binding to IgG-Sepharose (agg IgG). (B) Inhibition of  $0.5 \mu\text{g } ^{125}\text{I-sNP}$  binding by Bla mRF (○) and  $\gamma\text{-M anti-sNP}$  from L. serum (●).

fragment when coupled to Sepharose will bind Bla mRF as effectively as the ligand made with the whole IgG molecule. From the inability of monomeric IgG and the Fc fragment to precipitate with Bla mRF and analysis of the binding studies with monomeric IgG, only one antigen site appears to be present on each IgG molecule.

The nature of the DNA-protein reactive with Bla mRF was further characterized by gel diffusion and immunofluorescent studies. There was no precipitation in gel diffusion between Bla mRF and DNA, histone, or a preparation of the soluble nuclear antigens. Mixtures of DNA and histones also failed to give precipitation; however, preparations of nucleosomes precipitated with Bla mRF in gel diffusion. Polynucleosome preparations  $>19\text{S}$  precipitated as well as the DNA-nucleoprotein preparation, whereas oligonucleosomes and the  $11\text{S}$  mononucleosomes gave weaker lines. The role

of histones in the DNA-protein antigen could be confirmed by the ANF technique of Tan et al. (15). In this method, elution of tissue nuclei with 0.1 N HCl eliminates the reaction of the anti-DNA histone antibodies with the tissue nuclei. The reactivity can be reconstituted by treatment of the tissue with isolated histone (16). 0.1 N HCl treatment of tissue completely eliminates the ANF reaction of Bla mRF shown in Fig. 1 A. The results of reconstitution experiments are summarized in Table I. From these studies it appears that the histone fraction H2A + H2B is sufficient to reconstitute the nuclear antigen reactive with Bla mRF. Although the histone fraction H3 + H4 did not reconstitute, the possibility is not excluded that these histones in the nucleosome core are also reactive with Bla, because the presence of H3 and H4 in the reconstituted tissue was not confirmed in these experiments.

*Comparison of Interaction of Bla mRF with Monomeric IgG and Mononucleosomes.* From binding experiments of Bla with monomeric IgG, the valence of Bla mRF for monomeric IgG determined was 5 and the association constant ( $K_0$ ):  $3.75 \times 10^{-6} \text{ M}^{-1}$ . The comparable study of Bla binding with mononucleosomes could not be done because neither an active mononucleosome fragment with a single antigenic site nor an active Fab fragment of Bla could be prepared. However, because polynucleosomes could be radiolabeled without loss of reactivity with Bla mRF, inhibition by monomeric IgG and mononucleosomes could be compared in binding assays using either IgG or the DNA-nucleoprotein antigen as the substrate. The results are shown in Fig. 5. Where the binding of labeled nucleosomes by Bla was inhibited, the mononucleo-

TABLE I  
Characterization of Bla mRF Antinuclear Reaction by 0.1 N HCl Tissue  
Extraction and Reconstitution with Various Histone Fractions

	ANA Titer
Control tissue	$\geq 1,000$
Extracted tissue	0
Extracted tissue plus histone:	
Total	$\geq 1,000$
H1	0
H2a + H2b	$\geq 1,000$
H3 + H4	0

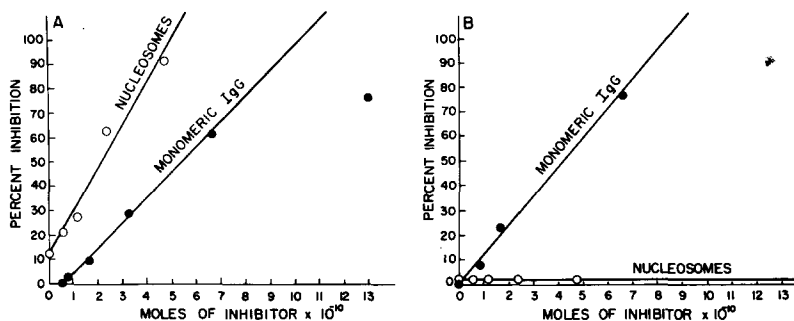


FIG. 5. Comparison of monomeric IgG (●) and mononucleosomes (200,000 mol wt) (○) inhibition. (A) Inhibition of  $1 \mu\text{g}$   $^{125}\text{I}$ -IgG binding by Bla mRF. (B) Inhibition of  $1 \mu\text{g}$   $^{125}\text{I}$ -polynucleosomes binding by Bla mRF.

some per mole gave greater inhibition than monomeric IgG. Where the binding of labeled monomeric IgG was inhibited, the mononucleosomes gave no inhibition.

*Occurrence of Polyclonal RF Cross-Reactive with DNA-Nucleoprotein.* Cross-reactivity similar to Bla mRF was demonstrated among polyclonal RF from a variety of pathologic sera. Because precipitin analysis in gel diffusion can be a relatively insensitive technique, two additional methods were used to assess the incidence of cross-reacting RF in sera in various diseases, blocking of ANF activity, or  $^{125}\text{I}$ -sNP binding by aggregated IgG. The incidence of cross-reactive RF which was found in various diseases using the three methods is shown in Table II. Approximately 40% of sera from seropositive classic rheumatoid arthritis (RA) contain cross-reactive RF. A high incidence is also found among the patients with the so-called rheumatoid overlap (RO) syndromes, but thus far, sera from RF-positive SLE patients have been negative. None of the RF from patients with mixed cryoglobulinemia tested were cross-reactive. Only a small group of mixed connective-tissue disease (MCTD) patients with positive RF tests were available for study; of these, in five of seven, the RF was cross-reactive. In two of these sera, the cross-reactive RF was detectable only in an isolated preparation of RF from the serum although the routine tests for RF were positive.

The RF of one of the positive MCTD sera available in large volume was isolated by IgG-Sepharose affinity chromatography in the same way Bla mRF was isolated. The precipitin reaction of the isolated Orl RF with DNA-nucleoprotein is shown in Fig. 6. DNase or trypsin digestion of the DNA nucleoprotein, as was the case for the Bla mRF reaction, eliminated the reaction with Orl. In contrast to Bla mRF, however, in the radioimmunoassay, the Orl polyclonal RF binding to aggregated IgG is not maximally inhibited by both aggregated IgG and DNA-nucleoprotein. Only ~25% of the Orl RF binding is inhibited by DNA-nucleoprotein.

Evidence that Orl and Bla reacted with similar antigenic determinants on the DNA-nucleoprotein is shown in Table III. Both Bla and Orl react with DNA-histone H2A-H2B complex.

*Characterization of BLA Cross-Idiotypic.* Characterization of the previously described WA cross-idiotype using the method of antigen binding inhibition is shown in Fig. 7. Anti-McD (anti-Ma in previous studies), which has specificity for the WA cross-idiotype antigen, inhibits the binding of Wa mRF, but no inhibition is obtained with anti-Lay, which has specificity for the PO cross-idiotype antigen or with anti-Bla. Bla

TABLE II  
*Incidence of Cross-Reactive RF*

	Number of RF-positive patients	Number of patients positive:			
		Gel diffusion	ANA*	NP binding‡	Overall positives
Classic RA	62	16	22	18	27
MCTD	7	5	5	5	5
Overlap syndromes	10	6	4	4	4
SLE	8	6	0	0	0
Mixed cryoglobulinemia	11	0	0	0	0
Miscellaneous	12	0	0	1	1

\* Blocked with aggregated IgG.

‡ NP, nucleoprotein. Blocked with aggregated IgG.

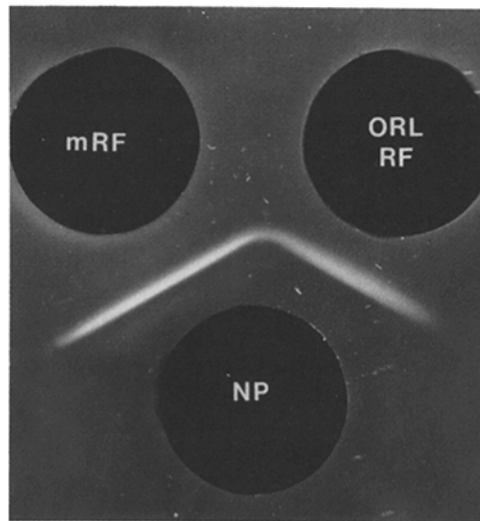


FIG. 6. 0.6% agarose gel diffusion plate. Precipitation of 1 mg/ml Bla mRF and 1 mg/ml OrI polyclonal RF with sNP.

TABLE III  
*Characterization of OrI Polyclonal RF Antinuclear Reaction by 0.1 N HCl  
 Tissue Extraction and Reconstitution with Various Histone Fractions*

	ANA titer
Control tissue	256
Extracted tissue	0
Extracted tissue plus histone:	
Total	64
H1	0
H2a + H2b	64
H3 + H4	0

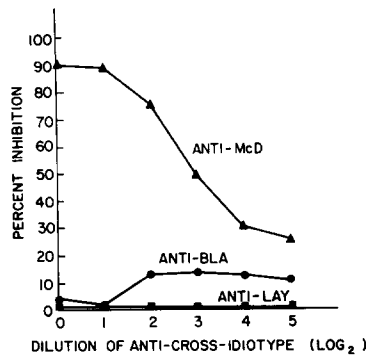


FIG. 7. Inhibition of 0.2 µg <sup>125</sup>I-Wa binding to IgG-Sepharose by dilutions of absorbed F(ab')<sub>2</sub> preparations of anti-McD (▲), anti-Bla (●), and anti-Lay (■). Starting concentration of antisera was that which gave maximal inhibition against the immunogen.



mRF was not inhibited by either anti-McD or anti-Lay and, hence, did not appear to belong to either the WA or PO groups. The anti-Lay used in these experiments was previously shown to inhibit Pom in the hemagglutination system (3). In the assay used here, this antiserum showed no inhibition of Pom binding to antigen, but gave strong inhibition of Lay binding to antigen. The same results were obtained with a second antisera raised to Lay.

The identification and isolation of another RF, Orl, with cross-reactivity similar to Bla mRF allowed studies to determine if a BLA cross-idiotype existed. Fig. 8A shows the inhibition by anti-Bla of the polyclonal Orl and four mRF that do not cross react with DNA-nucleoprotein, two of the WA group (Wa and McD) and two of the PO group (Pom and Lay). To be certain that the Orl inhibition was significant, each point was done in quadruplicate. Two standard deviations for each point were determined and compared to inhibition of Orl by anti-McD. The inhibition of Orl by anti-Bla was clearly significant by this analysis. Similar results were obtained with a second antisera to Bla raised in a rabbit and one raised in a goat. The results are consistent with the percentage of Orl RF binding to aggregated IgG that was inhibited

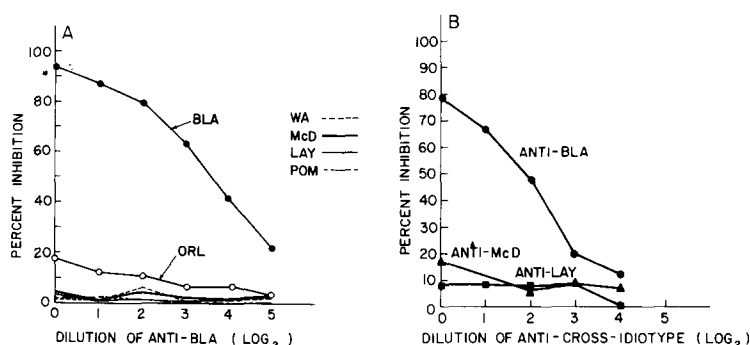


FIG. 8. Characterization of anti-Bla cross-idiotypic specificity by antigen-binding inhibition studies. (A) Inhibition of  $0.2 \mu\text{g}$  of various radiolabeled monoclonal RF binding to IgG-Sepharose by anti-Bla. (B) Inhibition of  $0.5 \mu\text{g}$   $^{125}\text{I}$ -polynucleosomes binding by Orl RF with dilutions of anti-Bla (●), anti-McD (▲), and anti-Lay (■).

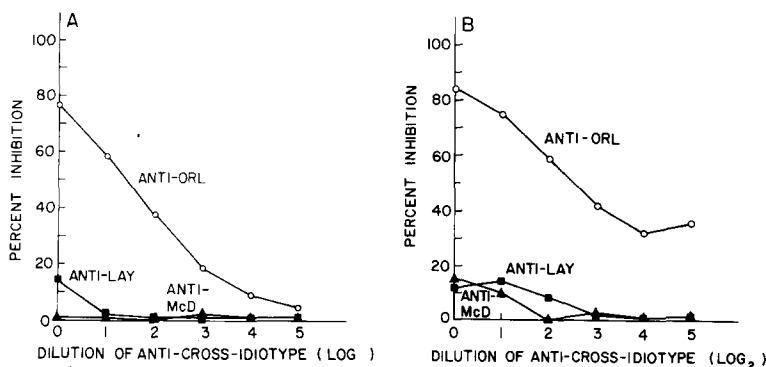


FIG. 9. Characterization of anti-Orl cross-idiotypic specificity by antigen-binding inhibition studies. (A) Inhibition of  $0.2 \mu\text{g}$   $^{125}\text{I}$ -Bla mRF binding to IgG-Sepharose by anti-Orl (○), anti-McD (▲), and anti-Lay (■). (B) Inhibition of  $0.5 \mu\text{g}$   $^{125}\text{I}$ -polynucleosome binding by Bla and mRF with anti-Orl (○), anti-McD (▲), and anti-Lay (■).

by DNA-nucleoprotein and indicate that only a portion of the Orl RF preparation bears the BLA cross-idiotype. That this is most likely the case is supported by studies of inhibition of Orl RF binding to nucleosomes (Fig. 8B). Whereas anti-Bla could inhibit only 18% of Orl aggregated-IgG binding, the same antisera could inhibit 78% of the Orl nucleosome binding. The existence of a BLA cross-idiotype was confirmed by an anti-Orl serum that inhibited Bla binding to aggregated IgG but did not inhibit the aggregate binding of the proteins of the WA and PO groups. Fig. 9A and B shows the studies defining the BLA cross-idiotype using this anti sera. Anti Orl inhibits Bla binding to both aggregated IgG and nucleosomes, whereas anti-McD, which defines the WA group, and anti-Lay which defines the PO group, give insignificant inhibition.

### Discussion

This study provides clear evidence for the cross-reactivity of an mRF, Bla, with DNA-nucleoprotein. In addition, evidence is present that the combining site of Bla mRF is involved in the cross-reactivity. This is the first monoclonal IgM anti- $\gamma$ -globulin studied to have this unique reactivity. Similar to the polyclonal RF cross-reactive with nuclei studied by Hannestad and Stollar (17), Bla mRF appears to react with an antigen on the nucleosome core which is known to consist of DNA and two molecules each of H2A, H2B, H3, and H4 (18). Our studies on the antigen reactive with Bla mRF indicate: (a) both DNA and histone are involved in the antigen determinant; (b) H2A + H2B when added to nuclei stripped of histone is sufficient to reconstitute the antigen; (c) the determinant is destroyed by trypsin treatment which is known to cleave 20–30 amino acid residues from the NH<sub>2</sub>-terminus of all four histones while leaving the remainder of the molecules and gross nucleosome structure intact (18). Because there are no known primary structural similarities between DNA histone and IgG which would explain the cross-reactivity, there must be some other type of molecular similarity. The finding that the antigenic determinant could be reconstituted by adding histone to nuclei extracted with 0.1 N HCl suggests that some retained organization of the DNA in the extracted nuclei is sufficient to orient the histones to produce the antigenic determinant.

The cross-reactivity of Bla mRF raises the question of the relationship of IgG and DNA-histone to the immunogen. Exhaustive attempts at answering this question by determining the  $K_0$  of Bla mRF for the IgG and DNA-histone antigen were unsuccessful because the  $K_0$  for DNA-histone needed could not be determined with the methodology employed. The  $3.75 \times 10^{-6} \text{ M}^{-1}$   $K_0$  obtained for monomeric IgG is low, like that of other RF and, therefore, uninformative. However, from the inhibition studies done, it could be concluded that the affinity of Bla mRF must be greater for IgG than for DNA-histone. These data suggest that neither IgG nor DNA-histone fit the combining site of Bla mRF sufficiently well to give high  $K_0$  and that of the two, DNA-histone gives the weaker reaction. It is possible that neither of these antigens is the immunogen and that their reactions with Bla mRF are the result of the presence of structures which coincidentally partially fit the combining site of this antibody. However, the occurrence of a somewhat analogous cross-reactivity, C1q interaction with both IgG and DNA, suggests that the phenomenon may not be just happenstance.

Some of these studies are similar to the studies on antibodies cross-reactive with menadione and 2,4-dinitrophenyl (19, 20) in that evidence is presented for the

specificity of the reaction of antibodies cross-reactive with two structurally unrelated antigens, IgG and DNA-histone. Additional evidence for the specificity of the combining site of Bla mRF for DNA-histone is that complement is activated by complexes of Bla and DNA-histone (R. Powell and V. Agnello. Unpublished observations.). Another manifestation of this biologic activity is that Bla can induce the lupus erythematosus (LE) cell reaction (7), which is known to require the fixation of complement after the reaction of antibody, with the DNA-nucleoprotein from disrupted nuclei.

Studies on Bla mRF, although of interest in their own right, take on greater significance with the finding reported in this study that similar cross-reactive polyclonal RF occur in a variety of human diseases. These findings confirm previous reports of RF in RA that cross-react with nuclear antigens (5, 21-23). The higher incidence found in our study may in fact be still higher. Our finding that some of these cross-reactive RF can be detected only after isolation on IgG-Sepharose has recently been confirmed by Johnson (23) who found the isolated RF in 14 of 15 random seropositive RA sera gave positive ANF reactions, and by Tipton et al. (24) who found 10 of 15 isolated RF had ANF activity. The broader screening done in our study indicates that the cross-reactive type of RF is not confined to RA but occurs in other diseases as well.

However, this type of RF does not invariably occur in all RF-positive sera as shown by the studies of idiopathic mixed cryoglobulinemia where no cross-reactive RF were found even after isolation studies on each serum. The cross-reactive RF also was not found in SLE and a number of miscellaneous seropositive diseases; however, isolation studies were not done in these groups; hence, their occurrence is not totally excluded.

The wide occurrence of polyclonal RF with cross-reactivity similar to the Bla mRF raised the possibility that these RF constituted a distinct subset. The finding that one representative isolated polyclonal RF, Orl, in comparative studies appeared to react with the same nuclear antigen as Bla mRF was consistent with this hypothesis. Additional evidence that these antibodies were related was obtained by cross-idiotype studies. It had previously been established that two cross-idiotype groups, PO and WA, existed among monoclonal anti- $\gamma$ -globulin and recently it has been reported that these and other cross-idiotypes were present among polyclonal RF (25, 26). Because a second monoclonal RF with DNA-histone cross-reactivity was not available, the demonstration of the BLA cross-idiotype was somewhat difficult because the study required use of a polyclonal RF, only a relatively small portion of which cross-reacted with DNA-nucleoprotein. The method which was found to best demonstrate cross-idiotype was direct inhibition of antigen binding by F(ab')<sub>2</sub> preparation of the absorbed typing antisera. By using this technique, it was possible to compare the inhibition of Bla and Orl binding to both IgG and DNA-nucleoprotein. Using three antisera raised to Bla, one to Orl and established WA and PO typing sera, clear evidence was obtained for a cross-idiotype antigen on Bla and Orl RF which was completely distinct from the WA and PO cross-idiotypes.

It should be noted that there appears to be some difference in the antigen-binding inhibition and the hemagglutination methods in defining cross-idiotype groups because the WA group could be defined by both techniques, whereas the PO group could be defined only by the hemagglutination method. It is possible that the antigen-binding inhibition technique defines only those idiotypes relating to combining site,

whereas the hemagglutination inhibition detects a broader range of cross-idiotypes which do not necessarily involve the combining site.

Previous studies on anti- $\gamma$ -globulins could not relate cross-idiotypes to the specificity of each anti- $\gamma$ -globulin for different subclasses of human IgG or rabbit IgG. In our study, there is a clear correlation between BLA idiotype and reactivity with the DNA-histone antigen. Thus far, none of the proteins in the WA and PO groups tested react with DNA-histone and none bore the BLA cross-idiotype. Capra and Kehoe (27) have postulated a relationship between cross-idiotypes, combining sites, and hypervariable regions based on the similarity of the amino acid sequences of the hypervariable regions of two of the proteins in the PO group, Lay and Pom. The lack of a known unique antigen specificity for the PO group limits, in part, the interpretation of those data. Amino acid-sequence studies currently being done on the Bla and Wa proteins when compared to the sequences already available on Pom and Lay may provide more specific information on relationship between cross-idiotype and antibody-combining site structure.<sup>2</sup>

The relationship if any of RF of different specificities to pathologic mechanisms or etiology in those diseases where they occur is unknown. From the studies presented here, one possible relationship is apparent. DNA-nucleoprotein has been demonstrated in various pathologic fluids from patients with RA (28). The presence of RF cross-reactive with DNA-nucleoprotein in such fluids could, therefore, have immunopathologic consequences through immune-complex formation.

### Summary

Cross-reactivity of a monoclonal rheumatoid factor with an antigen present on IgG and DNA-nucleoprotein was demonstrated, and evidence presented that the combining site of the antibody was involved in the reaction. The antigen on the DNA-nucleoprotein was shown to involve both DNA and histone fraction H2A + H2B and was trypsin sensitive. The relative binding affinity of the antibody appeared to be greater for IgG than the DNA-histone antigen.

Similar polyclonal cross-reactive rheumatoid factors were found in a variety of diseases. A high incidence was found among patients with rheumatoid arthritis and mixed connective tissue disease. None were detected in patients with systemic lupus erythematosus and idiopathic cryoglobulinemia. Studies on one representative isolated polyclonal rheumatoid factor demonstrated the same reactivity with DNA-histone H2A + H2B as the monoclonal antibody. Cross-idiotype studies using antigen-binding inhibition methods demonstrated the same cross-idiotype on the polyclonal and the monoclonal rheumatoid factor which reacted with DNA-histone. This cross-idiotype was shown to be distinct from the cross-idiotypes previously demonstrated on monoclonal IgM proteins with anti- $\gamma$ -globulin activity.

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<sup>2</sup> These studies are currently in progress in Dr. Capra's laboratory.

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