

## IDIOTYPIC CROSS-REACTIONS OF MONOCLONAL HUMAN LUPUS AUTOANTIBODIES\*

By YEHUDA SHOENFELD,<sup>‡</sup> DAVID A. ISENBERG,<sup>§</sup> JOYCE RAUCH,<sup>||</sup>  
MICHAEL P. MADAIG, B. DAVID STOLLAR, AND ROBERT S. SCHWARTZ

*From the Hematology-Oncology Division (Department of Medicine), New England Medical Center; the Department of Biochemistry and Pharmacology, Tufts University School of Medicine, Boston, Massachusetts 02111; and the Rheumatology Division, Montreal General Hospital, Montreal, Quebec H3G 1A4, Canada*

The origin of autoantibodies in systemic lupus erythematosus (SLE)<sup>1</sup> is unknown, and the mechanism that accounts for the diversity of serological abnormalities in this disease is poorly understood. Clues that may clarify these problems have been obtained by the use of hybridomas that produce monoclonal lupus autoantibodies, which permit analyses of individual antibodies (1-4). Most monoclonal examples of anti-DNA antibodies, the dominant type of lupus autoantibody, bind to different polynucleotides of varying base composition (5-8). These antibodies may thus recognize a common feature of the sugar-phosphate backbone of nucleic acids, and the diverse reactions of such antibodies with nucleic acids presumably depend on particular spacings of phosphodiester groups in the backbone. Phosphodiester groups occur in both polynucleotides and phospholipids, which can account for the ability of some monoclonal (8, 9) and serum-derived (10) lupus autoantibodies to bind to both species of molecules. In principle, therefore, a single lupus autoantibody could produce serological reactions with a variety of test antigens. Indeed, individual monoclonal lupus autoantibodies have been shown not only to bind to multiple nucleic acids, but also to produce the fluorescent antinuclear reaction, behave like the lupus anticoagulant, and react with cardiolipin, the antigen used in serological tests for syphilis (9).

In the present experiments we have continued our studies of monoclonal human lupus autoantibodies with an analysis of their idiotypes. The sharing of idiotypes by autoantibodies obtained from different patients would suggest that they are products of germ line genes that are dispersed throughout the population. We used three antiidiotypic antibodies to test 60 human anti-DNA monoclonal antibodies derived from seven SLE patients and found a substantial degree

\* Supported by grant AM 31151 from the National Institutes of Health.

<sup>‡</sup> Fellow of the American Fellowship, Inc.

<sup>§</sup> Supported by The Stanley Thomas Johnson Foundation.

<sup>||</sup> Fellow of the Arthritis Society of Canada.

<sup>1</sup> *Abbreviations used in this paper:* ds, double stranded; ELISA, enzyme-linked immunosorbent assay; HBS, hepatitis B surface antigen; PBS, phosphate-buffered saline; RIA, radioimmunoassay; SLE, systemic lupus erythematosus; ss, single stranded; TKP, 0.1 M potassium phosphate buffer containing 0.1% bovine serum albumin and 0.01% Tween-20 buffer; TPBS, PBS containing 1% Tween-20.

of idiotypic sharing not only among autoantibodies from the same patient, but also among autoantibodies from unrelated patients.

### Materials and Methods

*Human Monoclonal Anti-DNA Antibodies.* The clinical data of the seven patients with SLE whose lymphocytes were used for the preparation of human-human hybridomas (8) and the production and affinity purification of the monoclonal antibodies have been described previously (8, 11). All the monoclonal antibodies were IgM-k and were initially identified as anti-single-stranded (ss) DNA antibodies. Subsequent analyses also demonstrated their ability to bind to other polynucleotides (e.g., poly(dT) and poly(I)) and in some cases to cardiolipin (8). The antigen binding characteristics of the two monoclonal antibodies (B-16/6 and A-32/15) that were used to produce the antiidiotype antibodies are depicted in Fig. 1. These antibodies were derived from two unrelated patients. The source of the monoclonal antibodies is indicated by the prefix A, B, C, D, E, F, or G, which denote the seven patients.

*Production of Antiidiotype Antibodies.* Two antiidiotype sera (anti-32/15/R and anti-16/6/R) were prepared by monthly immunization of rabbits according to the multiple intradermal injection method of Vaitukaitis (12). For the first and second immunizations the monoclonal antibody was mixed with an equal volume of complete Freund's adjuvant (Difco Laboratories Inc., Detroit, MI). Thereafter, the immunizations were given in incomplete Freund's adjuvant (Difco). The rabbits were bled after 3 mo and 1 wk after each booster. Their sera were rendered idiotype specific by absorption on a human IgG-IgM Sepharose column. The rabbit sera were pumped through the Sepharose column in the cold for 18 h at a rate of 10–20 ml/h, which is equivalent to nine separate absorptions. The absorbed sera were tested by ELISA (8, 11) and RIA (5) methods for the presence of antiidiotypic activity by their ability to bind to the immunizing idiotype and a panel of human monoclonal and polyclonal immunoglobulins. A monoclonal mouse antiidiotype antibody (anti-32/15/M) was obtained from a hybridoma that was produced by fusing the nonsecreting myeloma cell line (P<sub>3</sub>x63-Ag.653) with spleen cells from a BALB/c mouse that was immunized with the monoclonal anti-DNA antibody A-32/15 according to a previously described method (1).

*Inhibition of Binding of Monoclonal Anti-DNA Antibodies to Antiidiotype Antibodies by Polynucleotides.* Antiidiotype sera were diluted 1:500 in 0.05 M borate buffer, pH 8, and 150  $\mu$ l was added to the wells of polystyrene plates, which were incubated overnight at 4°C. The antiidiotype coated plates were then washed three times with PBS containing 1% Tween-20 (TPBS) and then with PBS. Dilutions of ssDNA (0.39–50  $\mu$ g/ml) or poly(dT) (0.21–27  $\mu$ g/ml) were incubated with 1.0  $\mu$ g of B-16/6 or A-32/15 monoclonal anti-DNA antibody for 1 h at 37°C and then overnight at 4°C. The mixture was then transferred to the antiidiotype-coated plates and incubated for 2 h at 23°C. After three washes with TPBS and PBS, 150  $\mu$ l of goat anti-human immunoglobulin conjugated to alkaline-phosphatase was added and the plates were incubated overnight at 4°C. Determination of bound alkaline phosphatase was performed as described previously (11).

*Inhibition of Binding of Monoclonal anti-DNA antibodies to ssDNA by Antiidiotype Antibodies.* Dilutions of monoclonal anti-DNA antibodies (100  $\mu$ l) in 0.1 M potassium phosphate buffer containing 0.1% bovine serum albumin and 0.01% Tween-20 buffer (TKP) were mixed with 100  $\mu$ l of antiidiotype antisera or, as a control, normal rabbit serum and incubated in glass tubes for 1 h at 37°C. The mixture (75  $\mu$ l) was transferred to polystyrene tubes coated with ssDNA and incubated for 1 h at 37°C. The tubes were washed three times with TKP and 100  $\mu$ l of <sup>125</sup>I-affinity-purified rabbit anti-human IgM (100,000 cpm) was added. After overnight incubation at 4°C the tubes were washed three times with buffer and bound <sup>125</sup>I was measured in a  $\gamma$ -counter.

*Inhibition of Binding of Antiidiotype Antibodies to Their Homologous Idiotypes By Monoclonal Antibodies.* Affinity-purified monoclonal anti-DNA antibody was diluted in 0.05 M borate buffer, pH 8.6. Concentrations of monoclonal antibody that produced 50% of maximum binding to the homologous antiidiotype antibody were incubated in wells of polystyrene

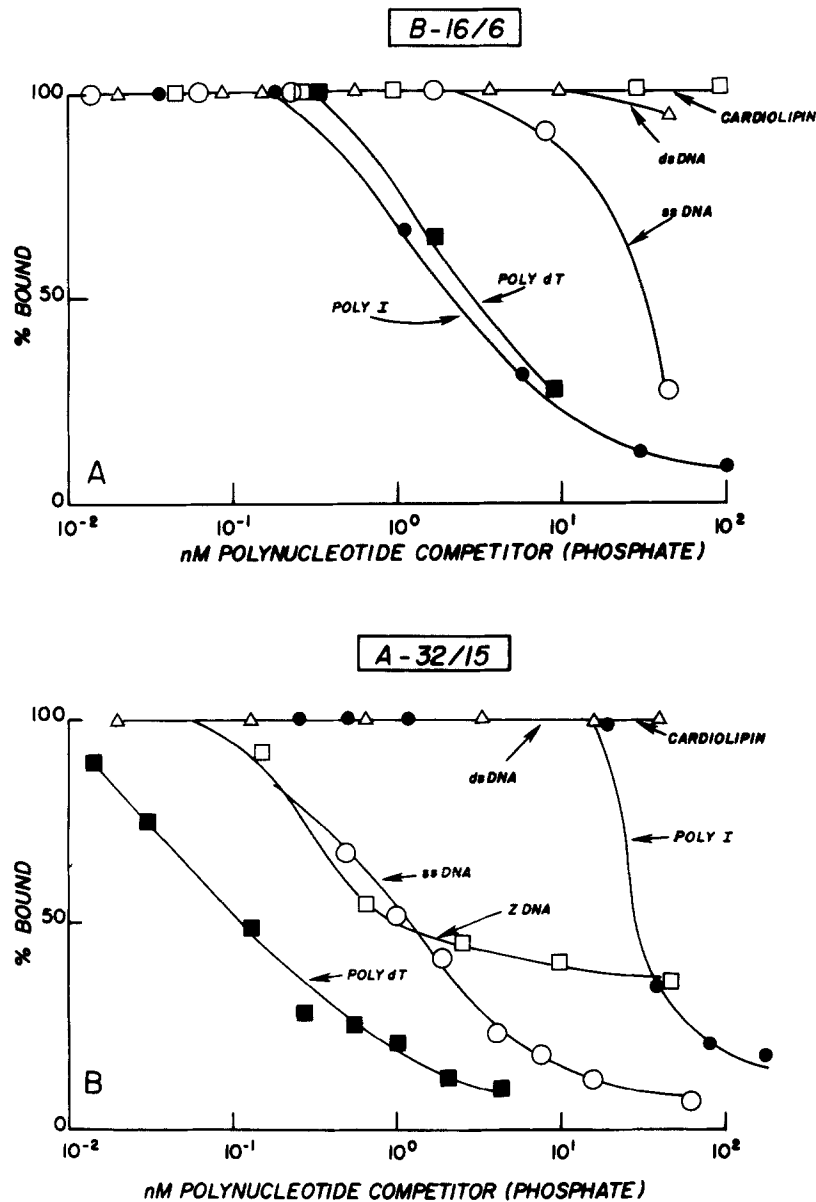


FIGURE 1. Ligand-binding characteristics of A-32/15 and B-16/6, the two monoclonal lupus autoantibodies that were used to prepare the antiidiotypic antibodies employed in these experiments. The autoantibodies were incubated with increasing amounts of various polynucleotides and then tested for residual binding to ssDNA (solid phase) by means of an ELISA method (8). Abscissa shows amount of competing polynucleotide and ordinate gives inhibition of the binding of the antibody to ssDNA.

plates overnight at 4°C. The idiotype coated plates were washed three times with TPBS and PBS. Equal amounts of diluted antiidiotypic antibody and dilutions of the competing monoclonal anti-DNA antibodies were incubated for 2 h at 23°C, and then applied to the idiotype-coated plates for 1 h. After three washes with TPBS and PBS, 150  $\mu$ l of goat anti-rabbit or rabbit anti-mouse immunoglobulin (for anti-32/15/M) conjugated to alka-

line phosphatase were added and the plates were incubated overnight at 4°C. The final steps of the assay were identical to those described above.

An analogous competitive assay was also carried out by RIA. Affinity-purified idiotypes were coated on polystyrene test tubes at a concentration of 0.25 µg/ml in 0.05 M sodium borate buffer, pH 8.6. Rabbit antiidiotypic antisera were used at dilutions that achieved ~50% of the maximum binding of antiidiotypic to tubes coated with homologous idiotypic. This dilution of antiidiotypic antiserum in TKP (100 µl) was mixed with 100 µl of various concentrations of affinity-purified competing monoclonal anti-DNA antibodies and incubated in glass tubes for 1 h at 37°C. The mixture (75 µl) was then transferred to idiotypic-coated tubes and allowed to incubate for 1 h at 37°C. The tubes were washed three times with TKP and 100 µl of <sup>125</sup>I-affinity-purified goat anti-rabbit immunoglobulin was added and the tubes were incubated overnight at 4°C. The tubes were then washed three times and bound <sup>125</sup>I was measured.

## Results

*Characteristics of the Antiidiotypic Antibodies.* Two antiidiotypic antisera were produced by immunization of rabbits with the monoclonal anti-DNA antibodies A-32/15 and B-16/6. The antiidiotypic antibodies, designated anti-16/6/R and anti-32/15/R, were used in solid phase (RIA or ELISA) assays in dilutions of 1:25,000 and 1:12,000, respectively. The monoclonal antiidiotypic (anti-32/15/M) was affinity purified on a goat-anti-mouse immunoglobulin Sepharose column and used in a dilution of 1:6,000 from a stock solution containing 300 µg/ml. Comparable results were obtained with these antibodies in independent tests conducted in Boston (ELISA) and Montreal (RIA). For simplicity, the results reported are those of ELISA assays, except where mentioned otherwise. The reaction of each antiidiotypic antibody with its idiotypic was 50% inhibited by 4–15 ng of the homologous idiotypic in competitive assays (see below). In both direct binding (Table I) and competition assays (see Fig. 3), none of the antiidiotypic antibodies reacted with two pools of affinity-purified IgM obtained from over 20 normal human donors, with five myeloma or Waldenstrom's proteins (both κ and λ) or with the IgGκ produced by the GM 4672 lymphoblastoid cell line that was used for the fusions. In addition, none of the antiidiotypic antibodies bound to ssDNA, dsDNA, or other polynucleotides (data not shown).

To test whether the antiidiotypic antibodies bound to a structure within or near the antigen-binding sites of the autoantibodies, we determined whether the

TABLE I  
*Optical Density (A<sub>405</sub>) Readings of Direct Binding Assays*

Test immunoglobulin	Antiidiotypic		
	Anti-16/6/R	Anti-32-15/R	Anti-32/15/M
Pooled normal IgM	0.02	0.06	0.04
A-32/15	—	1.00	0.34
B-16/6	1.00	—	—
Buffer only	0.01	0.01	0.01

Optical density (A<sub>405</sub>) readings of direct binding assays of the three antiidiotypic reagents used in the present experiments. The test immunoglobulins (1 µg/ml) were bound to polystyrene wells and then incubated with a 1:1,600 dilution of the antiidiotypes. Bound antiidiotypic was detected with alkaline phosphatase labeled goat anti-rabbit (or anti-mouse for anti-32/15/M) antisera. The final dilutions in which the antiidiotypes were used for the competitive assays shown in Table II and Figs. 3 and 4 were: 1:25,000 (anti-16/6/R), 1:12,000 (anti-32/15/R) and 1:6,000 (anti-32/15/M).

idiotype-antiidiotype reactions could be inhibited by prior incubation of the monoclonal autoantibodies with polynucleotides. Fig. 2 (*left*) depicts the inhibition of binding of A-32/15 to anti-32/15/M coated plates by poly(dT), poly(I), and ssDNA; 50% inhibition was achieved with 0.13  $\mu\text{g}$  poly(dT) and with 7.5  $\mu\text{g}$  poly(I). By contrast, up to 50  $\mu\text{g}$  ssDNA failed to cause 50% inhibition of the binding of A-32/15 to anti-32/15/M coated plates. The basis for the plateau in the competition of the monoclonal anti-idiotype antibody by ssDNA is not clear. The binding of A-32/15 to anti-32/15/R was 50% inhibited by 1.56  $\mu\text{g}$  ssDNA, 2.0  $\mu\text{g}$  poly(dT), and 3.8  $\mu\text{g}$  poly(I) (Fig. 2, *right*). Anti-16/6/R, however, was 50% inhibited only by poly(I) (23  $\mu\text{g}$ ).

*Cross-reactions of Monoclonal Autoantibody Idiotypes.* Multiple cross reactions were found among the monoclonal autoantibodies with the three antiidiotype reagents. Fig. 3 shows representative competition assays in which anti-16/6/R, anti-32/15/R and anti-32/15/M were employed. The results were selected from assays of 60 monoclonal anti-DNA antibodies to demonstrate cross-reactions that were observed with autoantibodies from the same patient, as well as with antibodies from unrelated patients (Table II). In Fig. 3A, for instance, the reaction of anti-16/6/R with B-16/6 was inhibited by monoclonal autoantibodies from patients A, B, and E. The similar contours of the competition curves observed with monoclonal immunoglobulins A-12/6 and B-16/5 suggests that anti-16/6/R detected similar idiotypic structures in these two autoantibodies. Fig. 3B demonstrates examples of competition curves obtained with anti-32/15/R (derived from patient A). Autoantibodies B-15/3 and F-101 generated almost parallel competition curves in this system, an indication that they possess closely related idiotypes. Examples of competition curves obtained with the monoclonal antiidiotype, anti-32/15/M, are given in Fig. 3C. Here, the strong cross-reaction with F-100 is notable; 32 ng of this antibody was required for 50% inhibition, a value comparable to the 15 ng required by A-32/15 itself. The reactions of a single monoclonal autoantibody, A-21/37, with the three antiidi-

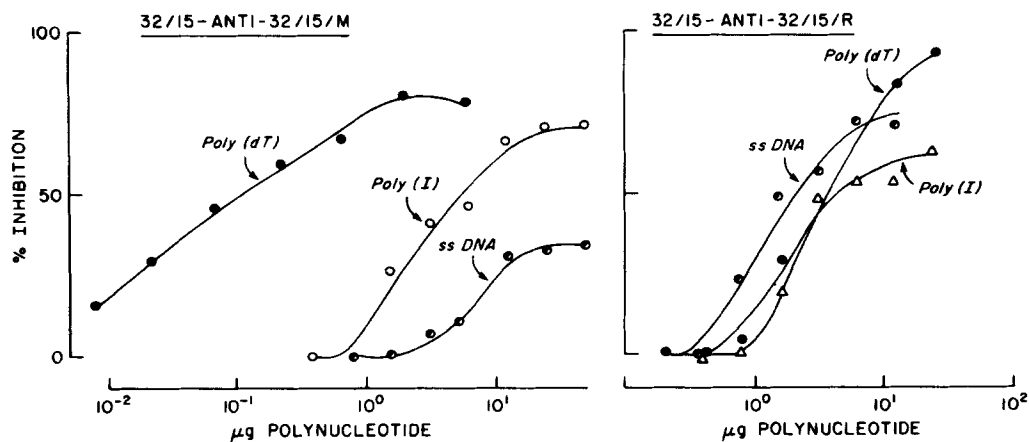


FIGURE 2. Inhibition of idiotype/antiidiotype binding by polynucleotides. The idiotype (monoclonal autoantibody) was incubated with increasing amounts of polynucleotide and then tested for residual binding to the corresponding antiidiotype (solid phase) by means of an ELISA method.

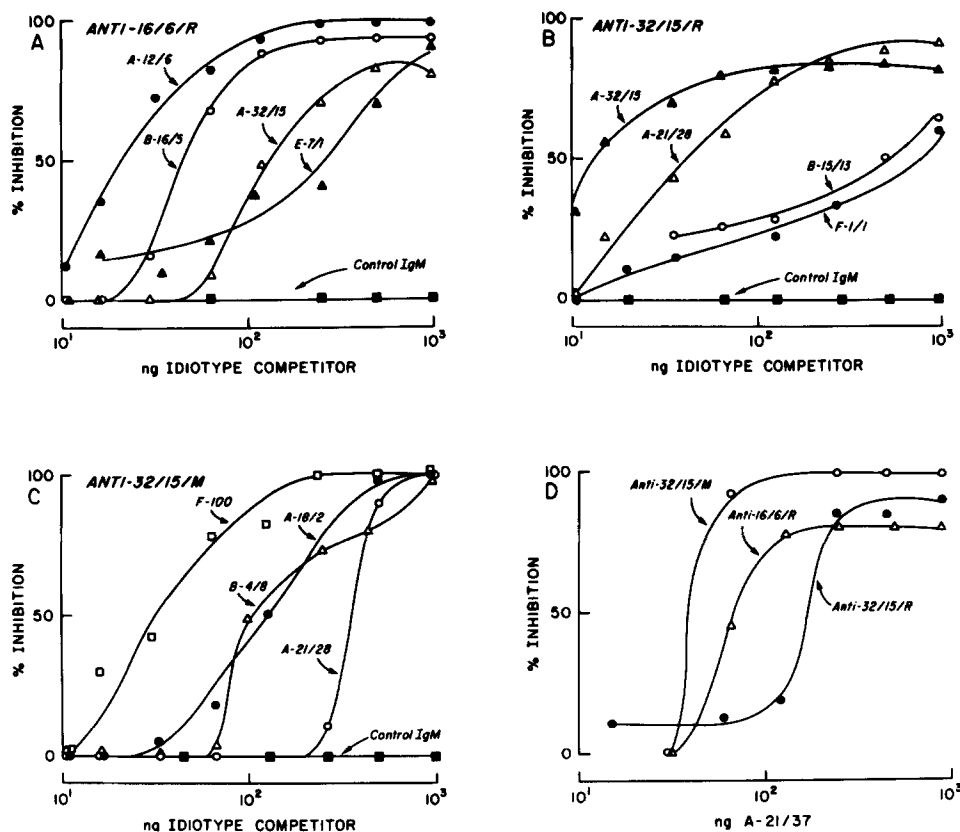


FIGURE 3. Representative solid phase competition assays of monoclonal autoantibody idiotypes. Increasing amounts of competing monoclonal antibody were incubated with the indicated antiidiotype reagent and the mixture was added to wells of polystyrene microtiter plates that were coated with the homologous monoclonal autoantibody. Bound antiidiotype was detected with goat anti-rabbit (for anti-16/6/R and anti-32/15/R) or rabbit anti-mouse (for anti-32/15/M) immunoglobulin by an ELISA method. (A) anti-16/6/R with B-16/6 on the solid phase; (B) anti-32/15/R with A-32/15 on the solid phase; (C) anti-32/15/M with A-32/15 on the solid phase; (D) reactions of A-21/37 in the three assay systems. "Control immunoglobulins" in panels A–C consist of two pools of affinity-purified IgM from over 20 normal persons, 5 myeloma and Waldenstrom's proteins and the IgG produced by the GM 4672 cell line. None of these immunoglobulins competed in the assays and results for all of them is shown by a single symbol.

otype reagents is shown in Fig. 3D. This antibody is one of 15 that cross-reacted with all three reagents. Table I lists the results for all 60 monoclonal autoantibodies, and in Fig. 4 the data are arranged to show the order in which the monoclonal autoantibodies cross-reacted in terms of the amount of immunoglobulin required for 50% inhibition. Of the 60 monoclonal autoantibodies, 20 failed to react with any of the three antiidiotype antibodies. Of the remaining 40, 30, derived from patients A, B, C, and E, reacted with anti-16/6/R (Fig. 4A); 22, from patients A, B, and C, reacted with anti-32/15/R (Fig. 4B); and 26, from patients A, B, D, E, F, and G, reacted with anti-32/15/M (Fig. 4C). Comparison of these groups demonstrates that the patterns of cross-reactivity with anti-32/15/M and anti-32/15/R differed. Another noteworthy feature is that 6 mono-

TABLE II  
*Results of Competitive Immunoassays*

Monoclonal antibody	Anti-16/6/R	Anti-32/15/R	Anti-32/15/M
A-1/9b	72	640	88
A-1/13b	4	15	98
A-1/17	3	20	NI
A-1/43	9	NI	NI
A-3/1	50	120	NI
A-9/1	NI	NI	NI
A-12/6	3	100	900
A-12/11a	3	700	54
A-12/33	3	110	65
A-18/2	6	100	NI
A-18/7	3	250	25
A-18/8	6	NI	NI
A-18/9	11	NI	NI
A-18/17	NI	NI	NI
A-21/28	520	22	245
A-21/29	87	22	13
A-21/37	56	140	37
A-30/17	100	190	NI
A-32/9	50	8	6
A-32/15	130	13	15
B-4/5	NI	250	40
B-4/7	6	NI	250
B-4/8	NI	NI	265
B-11/32	170	800	310
B-15/12a	500	540	680
B-15/13	54	320	NI
B-15/14	74	23	80
B-16/5	48	750	NI
B-16/6	4	440	250
C-4/1	NI	NI	NI
C-7/3	800	NI	NI
C-7/4	NI	NI	NI
C-7/9	NI	NI	NI
C-7/8	NI	NI	NI
C-12/1	70	NI	NI
C-12/2	NI	NI	NI
C-12/4	NI	NI	NI
C-12/9	31	10	NI
C-12/2	NI	NI	NI
D-7/9	NI	NI	NI
D-14/10	NI	NI	NI
D-20/5	NI	NI	195
D-36/12	NI	NI	NI
E-7/1	335	NI	960
E-14/10	120	NI	NI
E-19/10	300	NI	NI

TABLE II (Continued)

Monoclonal Antibody	Anti-16/6/R	Anti-32/15/R	Anti-32/15/M
F-100	NI	NI	32
F-100.1	NI	NI	NI
F-103	NI	NI	NI
F-103.1	NI	NI	NI
F-112.2	NI	NI	NI
F-113.3	NI	NI	370
F-121	NI	NI	195
F-128	NI	NI	440
F-134	NI	NI	300
F-134.1	NI	NI	NI
F-134.2	NI	NI	NI
G-600	NI	NI	NI
G-601	NI	NI	13
G-604	NI	NI	NI

Numerical values are the ng idiotypic competitor required for 50% inhibition of the homologous idiotypic-antiidiotypic reaction. *NI*, not inhibited by 1,000 ng.

clonal antibodies from patient A reacted at least as well with anti-16/6/R (derived from patient B) as B-16/6 itself (Fig. 4A).

Idiotypic cross-reactivity was also demonstrated by inhibition of the binding of monoclonal anti-DNA antibodies to ssDNA by anti-32/15/R or anti-16/6/R. Five monoclonal antibodies were chosen for this purpose (Fig. 5). The ability of the antiidiotypic antibodies to inhibit reactions of the autoantibodies with ssDNA was consistent with the ability of polynucleotides to inhibit idiotypic binding to antiidiotypic (Fig. 2), and again indicates that the antiidiotypic antibodies reacted with determinants in the autoantibodies that are required for polynucleotide binding. Nevertheless, the two test systems revealed differences: even though anti-16/6/R was able to block the binding of both B-16/6 and A-1/13b to ssDNA (Fig. 5), the binding of anti-16/6/R to B-16/6 was not inhibited by ssDNA.

### Discussion

We have demonstrated that IgM monoclonal anti-DNA autoantibodies derived from seven unrelated patients with SLE possess cross-reactive idiotypes. The antiidiotypic antibodies that detected these cross-reactions were produced by immunization of animals with IgM monoclonal antibodies derived from two unrelated patients. It is unlikely that the antiidiotypic reagents reacted with the Fc portion of the autoantibodies, or that they were antiallotypic antibodies, since they did not react with pooled immunoglobulins obtained from normal persons, nor did they bind to monoclonal immunoglobulins from patients with myeloma and macroglobulinemia. Instead, the antiidiotypic antibodies seemed to react with structures in or near the antigen combining sites of the monoclonal autoantibodies. This conclusion rests on the demonstration that binding of the monoclonal idiotypic to its homologous antiidiotypic antibody was inhibited by autoantigen (polynucleotide). The reaction of anti-32/15/M with A-32/15 was



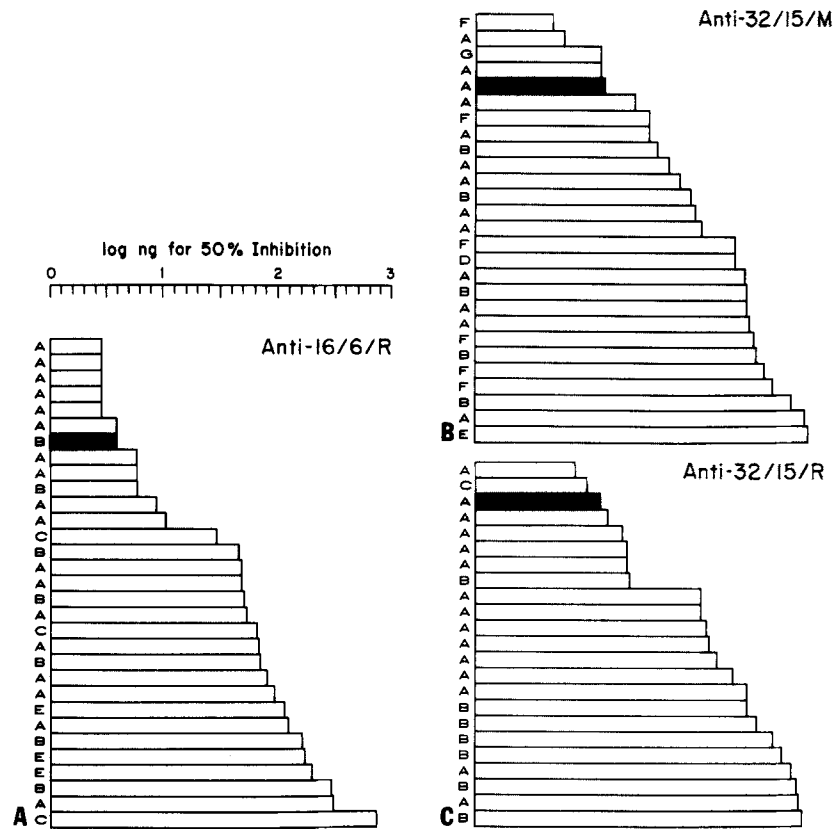


FIGURE 4. Results of the solid phase competitive assays shown in Fig. 3 for all cross-reacting monoclonal autoantibodies, arranged in order of the amount of autoantibody required for 50% inhibition of the homologous system. The black bar indicates the homologous autoantibody (e.g., B-16/6 for anti-16/6/R). Letters at the left of each bar denote the patient from whom the monoclonal autoantibody was derived. (A) anti-16/6/R with B-16/6 on the solid phase; 30/60 monoclonal antibodies, from patients A, B, C, and E, cross-reacted. (B) anti-32/15/R with A-32/15 on the solid phase; 22/60 monoclonal antibodies, from patients A, B, and C, cross-reacted. (C) anti-32/15/M with A-32/15/M on the solid phase; 25/60 monoclonal antibodies, from patients A, B, D, E, F, and G, cross-reacted.

almost completely inhibited by poly(dT) (0.13  $\mu\text{g}$  gave 50% inhibition) and poly(I) (3.8  $\mu\text{g}$  gave 50% inhibition), but <50% inhibition occurred with 50  $\mu\text{g}$  ssDNA (Fig. 2). Nevertheless, A-32/15 bound to all three polynucleotides, in the order poly(dT) > ssDNA > poly(I) (Fig. 1). It thus seems that the monoclonal antiidiotypic reacts with a relatively small "patch" in the variable region of A-32/15 that is required for binding to poly(dT) and poly(I), but not to ssDNA. The reaction of anti-32/15/R with A-32/15, by contrast, was inhibited by all three nucleic acids (Fig. 2B), albeit in a different order than they bound to A-32/15. Therefore, anti-32/15/M and anti-32/15/R probably react with different idiotypic epitopes in A-32/15. The inhibition results with anti-16/6/R are also of interest. The autoantibody B-16/6 bound to poly(I), poly(dT), and ssDNA (Fig. 1), yet its reaction with anti-16/6/R was inhibited only by poly(I). Even so, anti-16/6/R was able to block the binding of B-16/6 to ssDNA (Fig. 5). These

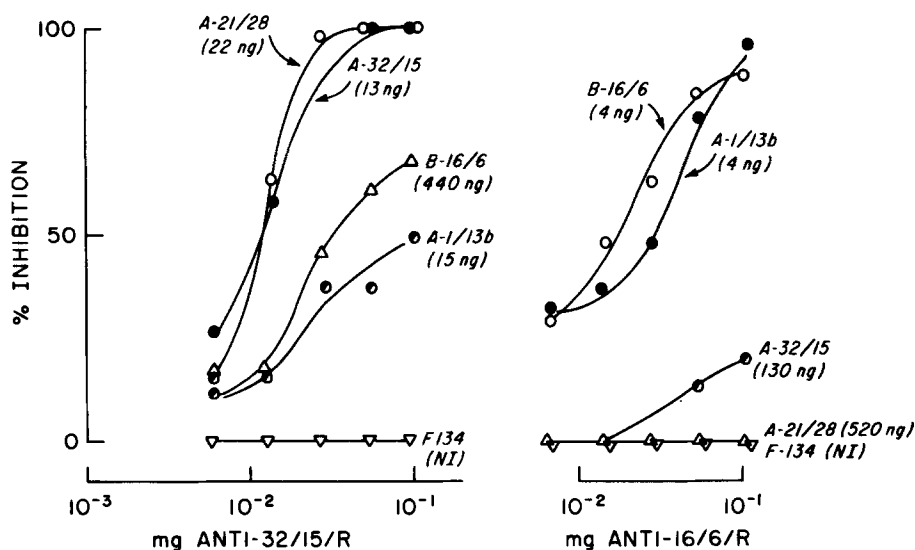


FIGURE 5. Inhibition of binding of idiotype (monoclonal autoantibody) to ssDNA by antiidiotype serum. Idiotype was added to increasing amounts of antiidiotype and the mixture was transferred to tubes coated with ssDNA. Bound idiotype was detected with <sup>125</sup>I-rabbit anti-human IgM. Numbers in parenthesis are the amounts of idiotype required for 50% inhibition in the assay for idiotypic cross-reaction shown in Fig. 3; *NI*, not inhibitory in that assay. Note that anti-32/15/R completely blocked the binding of A-32/15 to ssDNA (*left*) and that anti-16/6/R caused 90% inhibition of the binding of B-16/6 to ssDNA (*right*).

disparate results may reflect differences in the sensitivities of the various test systems, as well as the participation of different subsites in combining with antiidiotype or polynucleotides.

Of the 60 monoclonal autoantibodies we tested, 20 failed to react with any of the antiidiotypic reagents. These 20 antibodies had no binding properties with polynucleotides or cardiolipin that distinguished them from the other autoantibodies. Indeed, we found no relationship between antigen binding properties and idiotypic cross-reactions in the entire group (data not shown). Of the remaining 40 autoantibodies, 15 cross-reacted with one antiidiotype, 10 reacted with two antiidiotypes, and 15 cross-reacted with three antiidiotypes. The latter 15 presumably contain at least three serologically distinct idiotypic markers because anti-16/6/R, anti-32/15/R, and anti-32/15/M reacted with different variable region structures, as judged by the results of polynucleotide and idiotype inhibition studies (Figs. 2 and 4). Moreover, the observation that nine monoclonal autoantibodies derived from five patients reacted exclusively with anti-32/15/M (Table I) suggests that this reagent detected a unique idiotypic determinant. The ability of the monoclonal antiidiotype anti-32/15/M to detect a cross-reactive idiotype in autoantibodies from 6/7 patients is noteworthy because it indicates that a particular family of epitopes has a wide distribution among lupus autoantibody idiotypes. A similar result was recently reported by Solomon et al. (13) with a monoclonal antiidiotype obtained by immunization of a mouse with partially purified anti-DNA antibodies from the serum of a patient with SLE. This reagent detected a cross-reacting idiotype in the sera of 8/9 SLE patients.

Moreover, it reacted with anti-double stranded (ds) DNA antibodies of all IgG classes. Their antiidiotypic, unlike our reagents, is not directed against the antigen binding site because ds DNA does not inhibit its reaction with anti-ds DNA antibodies, nor does the antiidiotypic inhibit binding of anti-DNA antibodies to DNA. The reagent produced by Solomon et al. (13) can detect a cross-reactive idiotype in the serum of lupus patients with active clinical disease but without detectable anti-ds DNA antibodies. Such antiidiotypes may thus find useful clinical applications.

Anti-16/6/R reacted with 31 monoclonal autoantibodies derived from 4 patients (A, B, C, and E); anti-32/15/R reacted with 23 antibodies from 3 patients (A, B, and C) and anti-32/15/M reacted with 26 antibodies from 6 patients (Fig. 4). The occurrence of related idiotypic families in lupus autoantibodies from the seven patients is thus notable. These results support the hypothesis that the anti-DNA antibodies of SLE have a restricted diversity. They are also concordant with the above-mentioned restrictions that were found by analyses of the ligand binding properties of monoclonal lupus autoantibodies. Nevertheless, structural studies of the monoclonal autoantibodies, now in progress, will be required to define more precisely the basis of the idiotypic relatedness. Such studies are important because amino acid sequence analyses have demonstrated that considerable structural variations can exist even when monoclonal immunoglobulins possess closely related idiotypic markers (14).

Substantial idiotypic cross-reactivity occurs among monoclonal lupus autoantibodies derived from either (NZB  $\times$  NZW) $F_1$  (15, 16) or MRL-*lpr/lpr* mice (17), which may be due to their origins from inbred strains. Even so, Datta et al. (18) recently found that NZB mice can produce immunoglobulins that share idiotypes with a monoclonal anti-DNA antibody derived from genetically unrelated MRL-*lpr/lpr* mice. Cross-reacting idiotypes have also been reported with other human autoantibodies, including IgM cold agglutinins (19), rheumatoid factors (20), mixed cryoglobulins (21), anti-acetylcholine receptor antibodies from patients with myasthenia gravis (22), and cerebrospinal fluid immunoglobulins from patients with multiple sclerosis (23). In all these cases the autoantibodies were obtained from genetically unrelated persons.

Whether anti-DNA autoantibodies, or autoantibodies with other specificities, have any more idiotypic cross-reactions than other antibodies formed against a foreign antigen is not known. This possibility has not been extensively studied, but Kennedy and Dreesman (24) reported that antiidiotypic sera against purified anti-hepatitis B surface antigen (HBs) from two persons recognized an idiotype in purified anti-HBs from three additional individuals as well as in anti-HBs sera from six hemophiliacs. Thus, antibodies formed by humans in response to an infectious virus have shared idiotypes, an indication that extensively shared idiotypes are not unique to autoantibodies in man.

Public idiotypic markers of the type we found seem to be products of germline variable region genes (25). In the mouse, idiotypes of lupus autoantibodies are not confined to lupus-prone mice, but can also be produced by B lymphocytes of normal animals (18). This finding suggests that genes that encode the variable regions of lupus autoantibodies may exist in all mice, but only when there is a predisposition to SLE are they expressed. Whether this concept applies to human

SLE can now be tested because the appropriate antiidiotypic reagents are available. Finally, the occurrence of cross-reactive idiotypes in lupus autoantibodies, especially those that are detectable by a monoclonal antiidiotypic reagent, may render feasible the possibility of regulating the production of pathogenetic autoantibodies with antiidiotypic antibodies (26).

### Summary

Idiotypic cross-reactions were evaluated in 60 polynucleotide-binding monoclonal lupus autoantibodies produced by human-human hybridomas that were derived from seven unrelated patients with SLE. Three antiidiotypic reagents were prepared by immunization of rabbits or a mouse with monoclonal autoantibodies from two patients. Binding of the three reagents to their corresponding idiotypes was inhibited by one or more polynucleotides, an indication that the antiidiotypes reacted with the variable regions of the autoantibodies. Each antiidiotypic reagent appeared to detect a different idiotypic determinant. Of the 60 monoclonal autoantibodies tested, 40 reacted in one or more competitive immunoassays; 15 reacted with one antiidiotypic reagent, 10 reacted with two antiidiotypic reagents and 15 reacted with three antiidiotypic reagents. A monoclonal antiidiotypic reagent cross-reacted with autoantibodies from six of the seven patients. The idiotypic cross-reactions of immunoglobulins from unrelated patients suggest that the autoantibodies are derived from related families of germ line genes that are expressed by patients with SLE.

*Received for publication 28 March 1983 and in revised form 16 May 1983.*

### References

1. Andrzejewski, C. Jr., B. D. Stollar, T. M. Lalor, and R. S. Schwartz. 1980. Hybridoma autoantibodies to DNA. *J. Immunol.* 124:1499.
2. Hahn, B. J., F. Ebling, S. Freeman, B. Clevinger, and J. Davie. 1980. Production of monoclonal murine antibodies to DNA by somatic cell hybrids. *Arthritis Rheum.* 23:942.
3. Tron, F., D. Charron, J. F. Bach, and N. Talal. 1980. Establishment and characterization of a murine hybridoma secreting monoclonal anti-DNA autoantibody. *J. Immunol.* 125:2805.
4. Eliat, D., R. Asofsky, and R. Laskov. 1980. A hybridoma from an autoimmune NZB/NZW mouse producing a monoclonal antibody to ribosomal-RNA. *J. Immunol.* 124:766.
5. Andrzejewski, C. Jr., J. Rauch, E. M. Lafer, B. D. Stollar, and R. S. Schwartz. 1981. Antigen-binding diversity and idiotypic cross-reactions among hybridoma autoantibodies to DNA. *J. Immunol.* 126:226.
6. Pisetsky, D. S., and S. A. Caster. 1982. Binding specificity of a monoclonal anti-DNA antibody. *Mol. Immunol.* 19:645.
7. Koike, T., R. Nagasawa, N. Nagata, and T. Shirai. 1982. Specificity of mouse hybridoma antibodies to DNA. *Immunol. Lett.* 4:93.
8. Shoenfeld, Y., J. Rauch, H. Massicotte, S. K. Datta, J. Andre-Schwartz, B. D. Stollar, and R. S. Schwartz. 1983. Polyspecificity of monoclonal lupus autoantibodies produced by human-human hybridomas. *N. Engl. J. Med.* 308:414.
9. Lafer, E. M., J. Rauch, C. Andrzejewski, D. Mudd, B. Furie, B. C. Furie, R. S.

- Schwartz, and B. D. Stollar. 1981. Polyspecific monoclonal lupus autoantibodies reactive with both polynucleotides and phospholipids. *J. Exp. Med.* 153:897.
10. Koike, T., H. Tomioka, and A. Kumagai. 1982. Antibodies that cross-react with DNA and cardiolipin in patients with systemic lupus erythematosus. *Clin. Exp. Immunol.* 50:298.
  11. Shoenfeld, Y., S. C. Hsu-Lin, J. E. Gabriels, L. E. Silberstein, B. C. Furie, B. Furie, B. D. Stollar, and R. S. Schwartz. 1982. Production of autoantibodies by human-human hybridomas. *J. Clin. Invest.* 70:205.
  12. Vaitukaitis, J. L. 1980. Production of antisera with small doses of immunogen: multiple intradermal injections. *Methods Enzymol.* 74:46.
  13. Solomon, G., J. Schiffenbauer, H. D. Keiser, and B. Diamond. 1983. Use of monoclonal antibodies to identify shared idiotypes on human antibodies to native DNA from patients with SLE. *Proc. Natl. Acad. Sci. USA.* 80:850.
  14. Lamoyi, E., P. Estess, J. D. Capra, and A. Nisonoff. 1980. Heterogeneity of an intrastain cross-reactive idio type associated with anti-p-azophenylarsonate antibodies of A/J mice. *J. Immunol.* 124:2834.
  15. Marion, T. N., A. R. Lawton, J. F. Kearney, and D. E. Briles. 1982. Anti-DNA autoantibodies in (NZB × NZW)<sub>F1</sub> mice are clonally heterogeneous, but the majority share a common idio type. *J. Immunol.* 128:668.
  16. Tron, F., C. Guern, R. A. Cazenave, and J. F. Bach. 1982. Intrastain recurrent idiotypes among anti-DNA antibodies (NZB × NZW)<sub>F1</sub> hybrid mice. *Eur. J. Immunol.* 12:761.
  17. Rauch, J., E. Murphy, J. B. Roth, B. D. Stollar, and R. S. Schwartz. 1982. A high frequency idio typic marker of anti-DNA autoantibodies in MRL/*lpr/lpr* mice. *J. Immunol.* 129:236.
  18. Datta, S. K., D. Stollar, and R. S. Schwartz. 1983. Normal mice express idiotypes related to autoantibody idiotypes inherited by lupus mice. *Proc. Natl. Acad. Sci. USA.* 80:2723.
  19. Williams, R. Jr., H. G. Kunkel, and J. D. Capra. 1968. Antigenic specificities related to the cold agglutinin activity of gamma M globulins. *Science (Wash. D.C.)*. 161:379.
  20. Kunkel, H. G., V. Agnello, F. G. Joslin, and J. D. Capra. 1973. Cross-idio typic specificity among monoclonal IgM-proteins with anti-gamma-globulin activity. *J. Exp. Med.* 137:331.
  21. Geltner, D., E. G. Franklin, and B. Frangione. 1980. Anti-idio typic activity in the IgM fractions of mixed cryoglobulins. *J. Immunol.* 125:1530.
  22. Lefvert, A. K. 1981. Anti-idio typic antibodies against the receptor antibodies in myasthenia gravis. *Eur. J. Immunol.* 13:493.
  23. Tachovsky, T. G., M. Sandber-Wollheim, and L. G. Baird. 1982. Characterization of anti-idio type antibodies produced against MS CSF and detection of cross-reactive idiotypes in several MS CSF. *J. Immunol.* 129:764.
  24. Kennedy, R. C., and G. R. Dreesman. 1983. Common idio typic determinant associated with human antibodies to hepatitis B surface antigen. *J. Immunol.* 130:385.
  25. Weigert, M., and R. Riblet. 1978. The genetic control of antibody variable regions in the mouse. *Springer Sem. Immunopathol.* 1:133.
  26. Wigzell, H., H. Binz, H. Frischknecht, P. Peterson, and K. Sege. 1978. Possible role of auto-idio typic immunity in autoimmune disease. In *Genetic Control of Autoimmune Disease*. E. R. Rose, P. E. Bigazzi, and N. G. Warner, editors. Elsevier/North-Holland, New York. 322–338.