PARALLEL SETS OF AUTOANTIBODIES
IN MRL-lpr/lpr MICE
An Anti-DNA, Anti-SmRNP, Anti-gp70 Network

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In both human and murine lupus erythematosus, high-frequency public idiotypic markers occur on anti-DNA antibodies (1-4), yet Igs that do not bind to DNA can also possess the same markers (5, 6). This phenomenon, the sharing of idiotypic specificities by antibodies with different antigen binding properties, was originally described by Oudin and Cazenave (7) and is well established for antibodies to exogenous antigens (8, 9). It is probably due either to a V gene rearrangement in which a gene segment related to a particular idiotype is used for coding different Igs (10), or to a V gene mutation that affects the antigen-binding property of an antibody but not its idiotype (11). Antibodies with such recurrent idiotypes, termed parallel sets (12), are thought to underlie functional connections between B cell (or T cell) clones that respond to different antigenic determinants (13-15).

Parallel sets of antibodies could be important in autoimmunization. When examined in vitro, lymphocytes from patients with active lupus erythematosus produce Igs bearing the public idiotypic marker Id-16/6, a large fraction of which are anti-DNA antibodies. By contrast, only a small proportion of the Id-16/6+ Igs produced by cultured lymphocytes from patients with inactive lupus (or from normal subjects) bind to DNA (6). However, whether the remarkable shift in the antigen binding specificity of Id-16/6+ antibodies that occurs during relapse of lupus erythematosus relates to functionally connected parallel sets has not been established.

A public idiotype of anti-DNA antibodies also exists in MRL-lpr/lpr mice. This marker, termed Id-H130, occurs in 20-40% of the serum anti-DNA antibodies in that strain (3). And, analogous to the findings in human lupus, cultured lymphocytes from normal mice produce Id-H130+ Igs that do not bind to DNA, whereas a substantial fraction of the Id-H130+ Igs produced in vitro by MRL-lpr/lpr lymphocytes are anti-DNA antibodies (5). Even so, ~60% of the Id-H130+ antibodies in MRL-lpr/lpr serum do not bind to DNA (3). To identify those antibodies, we prepared hybridomas from MRL-lpr/lpr mice, screened them for Id-H130, and selected mAbs that did not bind to DNA. This report deals with a
remarkable example of such an antibody. It binds to SmRNP antigens and is an antiidiotyp against anti-DNA and anti-gp70 antibodies. This mAb appears to be the prototype of a population of similar antibodies in MRL-lpr/lpr serum. Our results suggest that a network of parallel sets of autoantibodies, with different autoantigen-binding properties, occurs in MRL-lpr/lpr mice.

Materials and Methods

Production of mAbs. Spleen cells were fused with the NS-1 plasmacytoma line and the desired hybridomas were cloned by limiting dilution (16). Pooled culture fluids from the hybridoma cell lines were affinity purified on Sepharose 4B coupled to goat anti-mouse immunoglobulins. The bound material was eluted with 0.1 M glycine HCl (pH 2.5), dialyzed against PBS (pH 7.4) and concentrated by ultrafiltration. The purified monoclonal antibodies TEPC 183, MOPC 195, MOPC 104, UPC 10 were purchased from Bionetics (Litton Bionetics Inc., Charleston, SC). MAb 28/12 was derived from an unimmunized 3 month old MRL-lpr/lpr mouse; MAb 512 and 319 are anti-DNA antibodies derived from unimmunized (SWR × NZB)F1 mice (17); mAb 514 is an IgM anti-retroviral gp70 antibody obtained from Dr. Miles Cloyd, Duke University Medical Center, Durham, NC (18); mAb 1417 is an IgM anti-retroviral gp70 antibody derived in our laboratory from a NFS mouse.

Production of Monoclonal Anti-Id H130. A BALB/c mouse was hyperimmunized with mAb H130 (2 × 106 hybridoma cells intraperitoneally biweekly three times, followed by 40 µg affinity-purified mAb H130 on days 5, 3, and 1 before fusion) and its splenocytes were fused with NS-1 cells. Hybridomas that produced antiidiotypes against mAb H130 were selected by a direct binding assay. For this purpose, microtiter plates were coated with either affinity-purified mAb H130 or TEPC 183 (0.5 µg/ml) in 0.05 M borate buffer (pH 8.6) for 18 h at 4°C. After blocking with 3% BSA in PBS (1 h at room temperature), supernatants that had been diluted 1:2 in diluting buffer (PBS, 1% BSA, 0.05% Tween) were added to the wells and incubated for 2 h at 37°C. The plates were washed once with 1% Tween in PBS and twice with PBS. Alkaline phosphatase-conjugated protein A (Zymed Laboratories, San Francisco, CA) was then added and the bound enzymic activity developed with p-nitrophenyl-phosphate. This procedure yielded a cloned hybridoma that produced mAb 108, an IgG1 antibody that bound specifically to mAb H130 in a liquid-phase competitive assay.

Id-H130 Assay. Production and characterization of the rabbit anti-H130 reagent has been described (19). The H130 idiotype (Id-H130) was detected by a competitive assay (19), with the following modifications: mAb H130 (precipitated twice from culture fluid by 50% ammonium sulphate and adjusted to a concentration of 1 mg/ml) was coupled to biotin according to Guesdon et al. (20) and used in an enzyme-linked immunoassay. Immulon 2 plates (Dynatech Laboratories, Inc., Alexandria, VA) were coated overnight at 4°C with 100 µl of the rabbit anti-H130 (1:2000) or anti-H130 (0.5 µg/ml) mAb in borate buffer. After blocking with 3% BSA in PBS for 1 h at room temperature, 50 µl of the biotin labeled mAb H130 (0.2 µg/ml) and 50 µl of the test antibody in diluting buffer were incubated together on the plate for 1 h at 37°C. The plates were washed once with 1% Tween in PBS, twice with PBS, and then avidin-alkaline phosphatase (Boehringer Mannheim Biochemicals, Indianapolis, IN) in diluting buffer was added. After 1 h at 37°C, the plates were washed and the bound enzymic activity was measured.

Anti-SmRNP Activity. SmRNP was affinity purified from rabbit thymus extract on a human anti-RNP Sepharose column. Briefly, the rabbit thymus extract was solubilized in 0.01 M phosphate, 0.35 M NaCl, 0.35 M NaCl, 2 mM PMSF (pH 7.4) by sonication and applied to the column. The column was rinsed with 0.01 M phosphate/0.5 M NaCl (pH 7.4); the bound antigen was eluted with 3 M NaSCN and immediately dialyzed against PBS. The eluate, which contains both RNP and complexed Sm antigen, was precipitated on ice with 20% TCA for 15 min, resuspended in 20 µl of sample buffer (0.12 M Tris-HCl, 20% glycerol, 6% SDS, 0.04 bromophenol blue, 10% 2-ME) and applied to a 10–20% acrylamide gradient gel. To define the specificity of SmRNP binding antibodies, the polypeptides separated by SDS-PAGE were transferred to nitrocellulose paper (21). The paper was
saturated with 5% BSA in PBS for 2 h at room temperature, then individual strips were incubated with the biotin-labeled test antibody in diluting buffer for 1 h at 22°C. After extensive washing with PBS-Tween 1%, the paper strips were incubated with alkaline phosphatase-conjugated avidin (Boehringer Mannheim Biochemicals) for 2 h at 22°C. The immunoactive bands were visualized using 5-bromo-4-chloro-indoxyl phosphate and nitroblue tetrazolium as the substrate (22). To measure anti-SmRNP activity, the eluate of the anti-RNP column (5 μg/ml in borate buffer) was used to coat polystyrene plates. After 18 h at 4°C, the plates were blocked by 3% BSA for 1 h and the antibodies or sera were added in diluting buffer to individual wells. The bound antibodies were detected by alkaline phosphatase-conjugated goat anti-mouse Ig.

**Antidiotypic Activity of mAb 28/12.** Polystyrene plates were coated with IgM mAbs (0.1 μg/ml) in borate buffer, and blocked with 3% BSA in PBS. For the direct binding assay, mAb 28/12 or control IgG antibodies were incubated in diluting buffer on the plate for 2 h at 37°C and detected by alkaline phosphatase-conjugated protein A. For the competitive assay, the amount of mAb 28/12 that gave 50% maximum binding to the test mAb was preincubated with different dilutions of mAb 1 h at 37°C and the mixture was transferred to the coated plates. The assay was then carried on as in the direct binding assay.

**Anti-Viral Activity.** Cloned polytropic retroviruses isolated in our laboratory (23) were propagated on mink lung fibroblasts (CCL64). Supernatants from fibroblasts infected with P1, a recombinant retrovirus, were applied to a Con A-Sepharose column (Pharmacia Fine Chemicals, Piscataway, NJ), eluted with α-methyl-D-mannoside (Sigma Chemical Co., St. Louis, MO) and dialyzed against PBS. After addition of NP40 (final concentration, 5%), the purified virus was applied to a Sepharose 4B-coupled anti-gp70 mAb (24). The unbound material was removed from the column by washing with 0.5% NP40-PBS and the gp70 was eluted with 0.1 M glycine (pH 2.5). After dialysis against PBS and concentration, the purity of the gp70 was authenticated by SDS-PAGE. The purified gp70 was used to coat polystyrene plates at a concentration of 1 μg/ml. A retrovirus-induced thymic leukemia line, provided by Dr. Hiroshi Hiai, Aichi Cancer Research Institute, Nagoya, Japan, was used for immunofluorescent flow cytometry. Briefly, the cells were washed twice in PBS/0.01% sodium azide; 2.5 μg of the antibody in 50 μl of PBS azide was added to pellets containing 10^6 cells. The pellets were resuspended, incubated on ice for 30 min, washed once with cold PBS-azide, and then incubated with 50 μl of a 1/60 dilution of FITC-conjugated goat anti-mouse IgM (Meloy Laboratories Inc., Springfield, VA). The cell pellets were washed again with PBS-azide and analyzed by cytofluorography. Membrane lysates from this cell line were used for immune precipitation, as previously described (24), with minor modifications. Briefly, 2 X 10^7 cells were radiolabeled with 1 mCi Na^125I and lactoperoxidase, washed with cold PBS, and lysed in 0.01 M Tris-HCl, 0.15 M NaCl, 0.5% NP40, and 2 mM PMSF (pH 7). The lysate was centrifuged to remove cellular debris and precleared twice with Staph A (IgG Sorb; The Enzyme Center, Inc., Boston, MA) coupled to rabbit anti-mouse IgM (Litton Bionetics, Kensington, MD) and once with Staph A alone. Protein A-Sepharose 6MB beads (Pharmacia Fine Chemicals) were coupled to rabbit anti-mouse IgM (Litton Bionetics) and incubated 2 h at 4°C. After washing with washing buffer (PBS 0.1% BSA, 0.5% NP40, 2 mM PMSF), 10 μg of the monoclonal IgMs were incubated with 50 μl of the packed beads for 2 h at 4°C. After washing, 2 X 10^6 cpm of the lysate were added to each tube in a final volume of 100 μl and incubated for 18 h at 4°C. The beads were washed five times with 5 ml of the washing buffer and twice with PBS. The immune precipitates were eluted by incubating in sample buffer for 3 min at 70°C and analyzed by SDS-PAGE on a 15% polyacrylamide gel. The dried gel was autoradiographed at -70°C with an x-ray intensifying screen.

**Results**

**Derivation of mAb 28/12.** MRL-lpr/lpr serum contains a population of Id-H130' anti-DNA antibodies as well as a second Id-H130' population that does not bind DNA (3). To analyze the specificities of the second population, we
prepared mAbs by first screening MRL-lpr/lpr hybridomas for Id-H130, and then for the lack of reactivity with DNA. mAb 28/12 fulfilled these two screening criteria. It was purified by an immunoaffinity column from the supernatant of a clone derived by limiting dilution from the original Id-H130+ 28/12 hybridoma line. The presence of Id-H130 in the purified antibody was established with both polyclonal and monoclonal antiidiotype reagents (Fig. 1). In liquid phase competition assays with the polyclonal antiidiotype, 50% inhibition was observed with 0.9 μg/ml of mAb 28/12, compared with 0.09 μg/ml of mAb H130, the prototypic Id-H130+ anti-DNA mAb (Fig. 1A). With the monoclonal antiidiotype, the amounts required for 50% inhibition were 5 μg/ml (mAb 28/12) and 2.2 μg/ml (mAb H130) (Fig. 1B). In a control assay with an unrelated idiotype/rabbit antiidiotype system, mAb 28/12 had no inhibitory effect in amounts up to 10 μg/ml.

*mAb 28/12 Is An Anti-SmRNP Antibody.* The ligand-binding properties of mAb 28/12 are shown in Figs. 2 and 3. The antibody did not react with single-stranded DNA, RNA, Poly (I), or cardiolipin. However, it did bind strongly to antigens in the SmRNP complex (Fig. 2). In an immunoblot assay, mAb 28/12 bound to a 49-kD polypeptide in the SmRNP complex; additional bands at 28, 18, 17, 13 kD, and a fainter band at 40 kD, were also observed (Fig. 3). The 28, 18, and 13 kD polypeptides are associated with the Sm antigen (25, 26). The 49-kD polypeptide, although present in the SmRNP complex (25), is not reactive with typical anti-Sm or anti-RNP antisera. The reaction of mAb 28/12 with antigens of the Sm-RNP complex was confirmed by affinity chromatography of the rabbit thymus extract with a mAb 28/12-Sepharose column. The eluate from this column, when analyzed by SDS gel electrophoresis, contained proteins with M₀ (× 10⁶) of 70, 58, 49, 43, 32, 20 and 13. These components have been
Antibody concentration (µg/ml)

**Figure 2.** Antigen-binding properties of mAb 28/12. Increasing amounts of mAb 28/12 were incubated on plates coated with SmRNP (5 µg/ml) or single-stranded DNA (2.5 µg/ml). The bound antibody was detected by alkaline phosphatase-conjugated goat anti-mouse Ig.

obtained by others in eluates of anti-Sm or anti-RNP Sepharose columns (25). The 43 kD protein is not part of the complex, since it binds to normal Ig columns. The 70 kD protein corresponds to the main antigen recognized by anti-RNP antibodies and the 13 kD protein corresponds to the E component of the Sm antigen. Polypeptides of MW 31-32 kD are associated with U1 RNA and react with anti-RNP or anti-Sm antisera (25).

**mAb 28/12 is an Antidiotypic Antibody.** To determine whether mAb 28/12 is a rheumatoid factor, we tested it against a panel of other mouse mAbs (Fig. 4). It bound to only 4 of the panel of 16 test antibodies. Those four antibodies did not bind to a control IgG2b mAb with the same allotype as mAb 28/12; therefore, they too were not rheumatoid factors. Of the four antibodies to which mAb 28/12 bound, two (mAb 512 and mAb 319) are anti-DNA mAbs derived from (NZB × SWR)F₁ mice (17); the other two (mAb 514 and mAb 1417) are anti-retroviral gp70 mAbs from (BALB/c × C57BL/6)F₁ (18) and NFS mice, respectively.

The specificity of the binding of mAb 28/12 to mAbs 512, 319, 514, and 1417 was confirmed by liquid-phase competitive inhibition assays. Table I shows the concentrations of various IgGs that were required to cause 50% inhibition of the binding of mAb 28/12 to the panel of four mAbs. In this checkerboard assay system, mAb 512 and 514 inhibited the binding of mAb 28/12 to all four test IgGs; mAb 1417 inhibited the binding of antibody mAb 28/12 to mAbs 512, 514, and 1417, but not to mAb 319; and mAb 319 inhibited the binding of mAb 28/12 to only mAb 1417. Four control mAbs did not inhibit any of the reactions of mAb 28/12. Additional evidence for the specificity of the binding of mAb 28/12 is shown in Table II, which lists the origins and isotypes of 28 mAbs that failed to inhibit the binding of mAb 28/12 to mAb 1417 in a liquid-phase competitive inhibition assay.

The data presented thus far show that mAb 28/12 is both an anti-SmRNP antibody and an antidiotypic antibody. To pursue the latter, antigen inhibition assays were carried out (Fig. 5). For this purpose, the two anti-DNA antibodies, mAbs 512 and 319, were first incubated with either single-stranded DNA or RNA and then transferred to plastic wells coated with mAb 28/12. With both
mAb 512 and mAb 319, single-stranded DNA, but not RNA, displaced the binding reaction; 50% inhibition of the 512-28/12 reaction occurred with 0.1 nM DNA (as phosphate), and 0.07 nM DNA (as phosphate) caused 50% inhibition of the 319-28/12 reaction. By contrast, neither gp70 nor whole retrovirus displaced the binding of mAb 28/12 from the two anti-gp70 mAbs, mAb 514 and mAb 1417 (data not shown).

The relationship between the anti-SmRNP activity and the antiidiotypic prop-
FIGURE 4. Binding of mAb 28/12 to mouse IgM. mAb 28/12 (open bars) or MOPC 195 (closed bars) at a concentration of 2 μg/ml were incubated on plates coated with 0.1 μg/ml of IgM and detected by alkaline phosphatase–conjugated protein A.

| Table I | Concentration of Ig Required for 50% Inhibition of the Binding of mAb 28/12 |
|---------|-----------------------------|
| Solid phase | Liquid phase |
| | 514 | 1417 | 512 | 319 | 183 | 104E | H130 | H62/1 |
| μg/ml | 0.1 | 2.5 | 0.08 | >10 | >10 | >10 | >10 | >10 |
| 1417 | 2.0 | 0.2 | 0.02 | 1.0 | >10 | >10 | >10 | >10 |
| 512 | 1.3 | 1.3 | 2.0 | >10 | >10 | >10 | >10 | >10 |
| 319 | 6.0 | >10 | 3.0 | >10* | >10 | >10 | >10 | >10 |

Each of the four IgMs was at a concentration of 0.1 μg/ml. The amount of mAb 28/12 that gave 50% of the maximum binding was preincubated with different quantities of the tested IgG and transferred to the plates. Bound mAb 28/12 was detected by alkaline phosphatase–conjugated protein A. Results are expressed as amount (μg/ml) of the tested Ig required to achieve 50% inhibition of mAb 28/12 binding. 183 is TEPC183, 104E is MOPC 104E, H62/1 is a monoclonal MRL-lpr/lpr IgM anti-single-stranded DNA antibody.

* The highest concentration of 319 tested (10 μg/ml) gave 25% inhibition.

| Table II | Isotypes and Strains of Origin of mAbs that Did Not Inhibit the Binding of mAb 28/12 to mAb 1417 |
|---------|----------------------------------|
| Mouse strain | Isotype | Specificity |
| BALB/c | IgM | TEPC 183, MOPC 104E, H248, H152, A71 |
| | IgG | UPC 10, MOPC 195, MOPC 21, J606 |
| NZB | IgM | H201, H525, H528, H603 |
| | IgG | H563, H567, H533, H550, H556, H549 |
| C57BL/6 | IgM | H50 |
| MRL-lpr/lpr | IgM | H130, H62/1 |
| | IgG | H225, H241, H147, H215 |
| A/J | IgG | H65 |
| NFS | IgG | H1416 |

mAb 512 has the NZB allotype (17) and mAb 514 was derived from a (C57BL/6 × BALB/c)F1 mouse.
PARALLEL SETS OF AUTOANTIBODIES

FIGURE 5. Inhibition of the binding of mAbs 512 and 319 to mAb 28/12 by DNA. Plates were coated with 0.5 µg/ml of mAb 28/12; the amounts of mAbs 512 or 319 required for 50% of the maximum binding to mAb 28/12 were preincubated for 1 h at 37°C with different amounts of single-stranded DNA or RNA and transferred to the plates. Bound antibody was detected by alkaline phosphatase–conjugated goat anti-mouse IgM. Results are expressed as percent inhibition. (Closed circles) mAb 319 + DNA; (open circles) mAb 319 + RNA; (closed triangles) mAb 512 + DNA; (open triangles) mAb 512 + RNA.

FIGURE 6. Inhibition of binding of mAb 28/12 to mAb 1417 by SmRNP. The eluate and the flow through of the anti-SmRNP column (SmRNP and rabbit thymus extract depleted of SmRNP, RTE-SmRNP) were tested for their ability to inhibit mAb 28/12 binding to mAb 1417. mAb 28/12 was preincubated with different amounts of SmRNP or RTE-SmRNP and transferred to plates coated with mAb 1417. Bound mAb 28/12 was detected by alkaline phosphatase–protein A.

In Fig. 6 it can be seen that the SmRNP antigen complex inhibited the binding of mAb 28/12 to mAb 1417. However, significant inhibition occurred only at a relatively high concentration of the SmRNP antigen (50% inhibition occurred at 70 µg/ml). Even so, the same concentration of the rabbit thymus extract had no inhibitory effect on the binding of mAb 28/12 to mAb 1417 after it was depleted of SmRNP antigens by immunoabsorption. In another assay, the
FIGURE 7. Inhibition of binding of mAb 28/12 to SmRNP by mAbs 514 and 1417. mAb 28/12 was preincubated with different amounts of mAbs 514, 1417 or TEPC 183 (control) and transferred to plates coated with SmRNP. Bound mAb 28/12 was detected by alkaline phosphatase–protein A.

reverse of the preceding, both mAb 1417 and mAb 514 in the liquid-phase completely displaced the binding of mAb 28/12 to solid-phase SmRNP (Fig. 7).

**mAbs 512 and 319 Bind to Both DNA and Retroviral Antigens.** The presence in mAbs 512, 319, 514, and 1417 of a shared idiotypic determinant recognized by mAb 28/12 prompted us to investigate whether these four antibodies also share an antigen-binding property. As the two anti-gp70 mAbs, mAb 514 and mAb 1417, did not bind to DNA, we sought evidence of gp70 binding by mAbs 319 and 512. Cytofluorography demonstrated that both mAb 319 and mAb 512 bound to an AKR thymic leukemia line that expresses gp70 on its plasma membrane. The component recognized by these two antibodies was further analyzed by immunoprecipitation of membrane lysates from ¹²⁵I surface-labeled cells (Fig. 8). The standard polyclonal and monoclonal anti-retrovirus antibodies immunoprecipitated 70 and 85 kD proteins from the cell lysates. These bands correspond to gp70 and either an 85 kD precursor of gp70 or a detergent-induced complex of gp70 and the retroviral protein p15 (27). mAb 512 also immunoprecipitated 70 and 85 kD proteins, whereas mAb 319 immunoprecipitated only the 85 kD protein.

We next tested the binding of mAb 512 to affinity-purified gp70. Purified retrovirus obtained from a cloned viral stock was applied to a monoclonal anti-gp70 Sepharose column (24) and the eluted 70 kD protein was used to coat polystyrene plates for binding assays. Fig. 9 shows the ability of mAbs 512, 514, and 1417 to bind to the affinity-purified gp70 (mAb 319 was negative in this assay). Taken together, these results show that mAb 512 is directed against gp70, whereas mAb 319 recognizes an epitope on the p85 complex that is either contained in the p15 moiety or in a determinant formed by the association of p15 and gp70.

**Antibodies Resembling mAb 28/12 in MRL-lpr/lpr Serum.** MRL-lpr/lpr sera were tested for anti-SmRNP antibodies that are also antidiotypes against mAb 514, the standard anti-gp70 mAb. Sera containing anti-SmRNP antibodies were pooled and passed through a pooled mouse Ig–Sepharose column to remove any
PARALLEL SETS OF AUTOANTIBODIES

Figure 8. SDS-PAGE analysis of cell surface proteins from an AKR thymic leukemia line. Immunoprecipitation was performed with: goat anti-AKR gp70 (lane 1), mouse monoclonal anti-gp70 (lane 2), MOPC104E (lane 3), mAb 512 (lane 4), mAb 319 (lane 5).

Figure 9. Binding of mAbs 1417, 514, 512, 319, and MOPC 104E to gp70. Different amounts of the mAbs were incubated on plates coated with 1 µg/ml of affinity-purified gp70 and detected by alkaline phosphatase–goat anti–mouse Ig. SDS-PAGE analysis of the gp70 is shown in the inset.

rheumatoid factors. The absorbed serum was next immunoaffinity purified on a mAb 514–Sepharose column. Antibodies in the eluate of this column bound specifically to mAb 514 and had anti-SmRNP activity (Fig. 10). Notably, anti-
DNA antibodies could not be detected in the eluate of the mAb-Sepharose column. The flow-through of the mAb 514-Sepharose column also contained anti-SmRNP activity.

Discussion

The experiments described in this paper identify a novel and theoretically important set of antibodies with relevance to the origin of autoantibodies against DNA and the SmRNP complex. The index antibody of the set, mAb 28/12, derived from a MRL-\(1pr/lpr\) mouse, was selected for detailed analysis because it bears a public idiotypic marker (Id-H130) of MRL-\(1pr/lpr\) anti-DNA antibodies, but fails to bind to polynucleotide antigens. Instead, mAb 28/12 is an anti-SmRNP antibody. It also reacts specifically with an idiotype in two anti-gp70 antibodies and in two anti-DNA antibodies. mAb 28/12, therefore, reveals an idiotypic network consisting of anti-DNA, anti-SmRNP, and anti-gp70 antibodies. Although we base this conclusion primarily on analyses of mAbs, such a network presumably occurs in vivo because antibodies similar to mAb 28/12 were found in MRL-\(1pr/lpr\) serum.

Anti-Sm antibodies occur in only ~25% of human lupus and MRL-\(1pr/lpr\) sera (28, 29), but they are specific for SLE (28, 30). Antibodies to single-stranded DNA, by contrast, occur in most lupus sera but they are not specific for that disease (31). The two kinds of autoantibodies are, therefore, considered to be unrelated. However, Pisetsky et al. (32) recently described an MRL-\(1pr/lpr\) mAb that binds to both single-stranded DNA and the Sm antigen, and that is idiotypically related to MRL-\(1pr/lpr\) anti-single-stranded DNA antibodies. mAb 28/12 is a notable variant of that kind of antibody. Although it shares an idiotype with mAb H130, a prototypic anti-DNA antibody, it is not an anti-DNA antibody but an anti-SmRNP antibody. mAbs 28/12 and H130, therefore, represent an
example of parallel sets of autoantibodies against different autoantigens. Pisetsky and Carter (33) found that an anti-Sm mAb and a mAb against a different antigen in rabbit thymus extract shared the same idiotype. Recently, Kaburaki et al.\(^1\) have shown that some human anti-DNA and anti-SmRNP antibodies share Id-16/6, a public idiotype that was originally identified in anti-DNA antibodies (2). Thus, idiotypic links between anti-DNA and anti-SmRNP antibodies occur in both human and murine lupus. If, as we propose, these two systems of autoantibodies are indeed parallel sets, then they might be functionally related (14). We may speculate that the down regulation of anti-SmRNP antibodies by a parallel set of anti-DNA antibodies could account for the relatively low frequency of anti-SmRNP antibodies in human and murine lupus (28, 29).

Brennan et al. (34) were able to prolong life in MRL-lpr/lpr mice by repeated administration of an anti-Sm mAb, yet serum levels of antibodies to double-stranded DNA were unaffected. However, antibodies to single-stranded DNA were not measured in their experiments.

The fine specificity of mAb 28/12 as an anti-SmRNP antibody differs somewhat from the binding properties of previously described anti-SmRNP antibodies. When used as an immunoabsorbent, mAb 28/12 removes from rabbit thymus extract a group of polypeptides, most of which have molecular masses identical to antigens of the SmRNP complex. In immunoblots, mAb 28/12 reacted with 49, 28, 18, 17, and 13 kD polypeptides. Anti-Sm mAbs can react with multiple low molecular mass polypeptides (35, 36), which could be either degradation products of higher molecular mass structures or distinct molecules that share one or more epitopes. However, no previously described anti-Sm or anti-RNP mAb reacts with a 49 kD polypeptide, as shown here with mAb 28/12.

Another interesting property of mAb 28/12 is its behaviour as an antidiotypic antibody. The binding of mAb 28/12 to other Iggs cannot be explained by rheumatoid factor or anti-allotypic activity. Of a total of 32 mAbs tested in various systems (Tables I and II), mAb 28/12 bound to only 4. Moreover, its binding to two anti-DNA antibodies (mAbs 512 and 319) was displaced by DNA, whereas a similarly charged nucleic acid, RNA, had no such effect. The antigen-specific displacement of mAb 28/12 from mAbs 512 and 319 indicates that their antigen-binding sites contain the idiotope recognized by mAb 28/12. The idiotope in the two anti-gp70 antibodies, mAbs 514 and 1417, may reside in their framework regions, since neither gp70 nor intact retrovirus inhibited their binding to mAb 28/12. We do not yet know whether the idiotope shared by these four mAbs is Ig chain–specific, or whether it is an antigenic surface formed by both the H and L chains. Nevertheless, it is interesting to consider that the framework region of one antibody could share an idiotope with the antigen-binding region of another antibody. Idiotope sharing by different Ig domains has been found in the variable and constant regions of rheumatoid factors (37) and is due to a recurrent tripeptide sequence in the two regions of the molecule (38).

A striking aspect of the four mAbs that mAb 28/12 recognizes is that all of them are anti-retrovirus antibodies. mAbs 514 and 1417 have been shown

\(^1\) Kaburaki, J., and B. D. Stollar. Identification of human anti-DNA, anti-RNP and anti-Sm serum antibodies bearing the cross reactive 16/6 idiotype. Analysis of sera from Japanese patients. Submitted for publication.
previously (18) to bind specifically to the gp70 of recombinant leukemogenic viruses. mAb 512 was shown here to bind to the gp70 of a cloned leukemogenic retrovirus and mAb 319 binds to either the gp70-p15 complex of the retroviral envelope or to p15 itself. These results are of even further interest because mAbs 512 and 319 are also typical anti–single-stranded DNA autoantibodies that react with poly(I) and Z-DNA (mAb 319), and poly(dT) and poly(I) (mAb 512) (17). Their ability to bind gp70 was investigated only after we found that mAb 28/12 is an idiotypotype against two known anti-gp70 antibodies, mAbs 514 and 1417. Thus, gp70 will have to be added to the list of antigens with which anti-DNA autoantibodies can crossreact, a list that includes cytoskeleton proteins (39), the Fc portion of IgG (40, 41), cell membrane proteins (42, 43), phospholipids (44), and bacterial polysaccharides (45).

mAb 28/12, as well as the anti-(anti-gp70) antibodies with anti-SmRNP activity we found in MRL-\textit{lpr}/\textit{lpr} serum, has the general properties predicted by Plotz (46); i.e., anti-(anti-viral) antibodies with autoantibody activity. Bunn et al. (47) have discussed the possibility that autoantibodies of the PL-12 type, which react with a cellular protein as well as certain tRNAs, are antiidiotypes of anti-viral antibodies. Plotz’s idea implies that pathogenetic autoantibodies can originate from perturbations of networks of antibodies against exogenous antigens. In principle, our results support his hypothesis. However, the anti-gp70 antibodies in the system we studied are probably not induced by exogenous infectious particles. On the contrary, they are more likely to represent autoantibodies against the endogenous gp70 that is produced from early life in MRL-\textit{lpr}/\textit{lpr} mice (48, 49).

Our results suggest that the anti-gp70, anti-DNA, and anti-SmRNP antibodies of MRL-\textit{lpr}/\textit{lpr} mice constitute a network of idiotypically related autoantibodies. The autoantibodies in this network seem to have a high degree of complementarity, or mimicry. Antiidiotypes against an IgG anti-DNA mAb have been found in the serum of (NZB × NZW)\textit{F}$_{1}$ mice by Eilat et al. (50), who also isolated two monoclonal examples of such antiidiotypes. Lymberti et al. (51) and Monestier et al. (52) have found extensive idiotypic relatedness among the natural autoantibodies produced by normal mice. Many of those antibodies bind to DNA (53). Mutual V region–dependent reactivity also occurs in high frequency among IgM mAbs derived from neonatal mice (54). These aboriginal antibodies of the preimmune repertoire thus resemble, in both their idiotypic organization and ligand-binding properties, the autoantibodies of lupus. It seems plausible, therefore, that the autoantibodies of this disease arise by expansion of a pool of idiotypically related, germline-encoded antibodies with anti-self reactivity. Our data suggest the hypothesis that some anti-SmRNP antibodies arise by mutation of a V gene segment that encodes anti-DNA antibodies. Such a process could account for the sharing of idiotypes by anti-SmRNP and anti-DNA antibodies (shown here), the ability of a mAb to bind to both SmRNP and DNA (32), as well as the random occurrence of anti-SmRNP antibodies in MRL-\textit{lpr}/\textit{lpr} mice, all of which produce anti-DNA antibodies (28). Structural studies of such autoantibody pairs, now in progress, should reveal the basis of their observed serological relationship.
Summary

The public idiotype Id-H130 occurs in MRL-\textit{lpr/lpr} serum both on a high proportion of anti-DNA autoantibodies as well as on antibodies that do not bind to DNA. To define members of the latter population, we prepared hybridomas and selected Id-H130\textsuperscript{+} mAbs that did not bind to DNA. One such antibody, mAb 28/12, was found to be an anti-SmRNP antibody. To determine whether mAb 28/12 had rheumatoid factor activity, we tested its ability to bind, in a solid-phase assay, to 16 mouse IgM mAbs. mAb 28/12 bound to only four of the panel, two anti-DNA antibodies (mAbs 512 and 319) and two anti-gp70 antibodies (mAbs 514 and 1417). In a liquid-phase competition assay with a panel of 32 monoclonal IgM and IgG antibodies, including allotype-matched Isgs, mAb 28/12 reacted only with mAbs 512, 319, 514, and 1417. The binding of mAb 28/12 to mAbs 512 and 319 was displaced by DNA, but not by RNA, indicating that the idiotype it defines (Id-28/12) is in the antigen-binding region of the two anti-DNA antibodies. In the two anti-gp70 antibodies (mAbs 514 and 1417), Id-28/12 seems to occur in the framework region. To determine if all four Id-28/12\textsuperscript{+} antibodies shared a common antigen-binding property, they were tested for their ability to react with DNA and gp70. The two anti-gp70 antibodies did not bind to DNA. However, the two anti-DNA antibodies were found to immunoprecipitate viral proteins from retrovirus-infected cells. mAb 512 reacted with gp70, both in cell membrane lysates and in purified form; mAb 319 reacted with gp85, which contains both gp70 and the retroviral protein p15. Antibodies with properties similar to those of mAb 28/12 were found in MRL-\textit{lpr/lpr} serum. It was possible, by affinity chromatography on an anti-gp70 antibody column, to isolate from serum those anti-(anti-gp70) antibodies with anti-SmRNP activity. These results show that parallel sets of autoantibodies, which share a common idiotype, but which bind to different autoantigens, occur in MRL-\textit{lpr/lpr} mice. Some populations of anti-DNA, anti-SmRNP, and anti-gp70 antibodies appear to constitute a network of autoantibodies in that strain. We speculate that part of the anti-SmRNP population of autoantibodies can arise by mutation of germine-encoded anti-DNA antibodies.

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