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Immunology

Normal mice express idiotypes related to autoantibody idiotypes of lupus mice

(monoclonal anti-DNA autoantibodies/shared idiotypes/autoimmunity)

SYAMAL K. DATTA*, B. DAVID STOLLAR†, AND ROBERT S. SCHWARTZ*

*Hematology/Oncology Division, New England Medical Center, and †Department of Biochemistry and Pharmacology, Tufts University School of Medicine, Boston, Massachusetts 02111

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ABSTRACT **Spleen and fetal liver B cells of normal mice synthesized idiotypes shared by anti-DNA autoantibodies of genetically autoimmune mice. Some of the idiotypes were specific for DNA; the majority, however, were not. The findings indicate that the autoantibody idiotypes are related to a conserved family of antibody variable regions that are present in normal animals.**

The spontaneous production of autoantibodies to DNA is characteristic of human and murine systemic lupus erythematosus (1, 2). These antibodies have been implicated in the pathogenesis of nephritis and other lesions of the disease (1, 2). Their production, however, is not restricted to mice that develop systemic lupus erythematosus. Strains of normal mice can spontaneously produce such antibodies in old age (3) or upon stimulation of their B cells by the polyclonal activator lipopolysaccharide (LPS) (4, 5). These observations have an important bearing on the origins of anti-DNA autoantibodies. Do the autoantibodies produced by autoimmune strains differ from their counterparts in normal strains? Are the same anti-DNA antibody-producing clones of cells present in both autoimmune and normal strains? We addressed these questions by determining if normal mice can produce antibodies with idiotypic markers of the anti-DNA autoantibodies of MRL-*lpr*/*lpr* (MRL/*lpr*) mice, a strain that develops a lethal form of systemic lupus erythematosus (1). Three idiotypic markers, designated H130, H102, and H43, were studied. These autoantibody variable (V) region markers were previously identified by rabbit anti-idiotypic sera that are specific for the antigen combining sites of the corresponding MRL/*lpr*-derived monoclonal anti-DNA antibodies (6). All MRL/*lpr* mice produce large amounts of H130, which is a prominent idiotype of the anti-DNA autoantibodies of that strain. H102 and H43 are produced in smaller amounts and their serum concentrations do not rise during the course of the autoimmune disease (7).

MATERIALS AND METHODS

Animals and Antibodies. All mice came from The Jackson Laboratory. Establishment of the monoclonal anti-DNA antibody-producing hybridomas H130, H102, and H43 from fusions of MRL/*lpr* spleen cells, purification of the antibodies, and production of the rabbit antisera specific for their idiotypes were previously described (6–8). The anti-idiotypic antibodies were prepared by absorption of the rabbit antisera successively on the following affinity columns: purified mouse IgM-IgG-Sepharose, Sepharose-bound globulins of MPC-11 ascites fluids, and Sepharose-bound globulins of pristane-induced (AKR × DBA/2)F_1 ascites fluid. The pooled flow-through fractions were repeatedly absorbed on the columns until the acid eluates (0.5 M acetic acid) from each column had an A_{280} less than 0.001. The absorbed anti-idiotypic reagents were further purified on a goat anti-rabbit IgG-Sepharose column with acetic acid as the eluting agent. The three anti-idiotypic antibodies were found to be specific for the respective monoclonal anti-DNA antibody idiotypes (H130, H102, and H43) and they did not crossreact with other myeloma proteins (refs. 6 and 7 and Fig. 1). Moreover, the binding of these antibodies to their respective idiotypes could be blocked by DNA and other polynucleotide antigens, suggesting that the anti-idiotypic antibodies were directed against the antigen combining sites of the idiotypes (6, 7). Pooled normal rabbit serum (NRS) and the goat anti-rabbit immunoglobulin (GαRIg) underwent the same purification and absorption steps as the anti-idiotype sera (6). All sera were adjusted to protein concentrations of $\approx$7 mg/ml.

Biosynthetic Labeling, Immunoprecipitation, and NaDodSO$_4$/Polyacrylamide Gel Electrophoresis. Fetal liver cells from 15- to 18-day-gestation embryos and adult spleen cells were prepared and cultured for 5 days with or without LPS (from *Salmonella typhosa* 0901) as described (9). Those cells, freshly prepared spleen cells (9), and hybridoma lines were washed twice in ice-cold methionine-free medium (10); 10^7 viable cells (5×10^6 in the case of hybridomas) were cultured in 1 ml of medium with 100 μCi (1 Ci = 3.7×10^{10} Bq) of [^{35}S]methionine (New England Nuclear) for 4 hr. After culture, cells were sedimented and the culture supernatants were placed in centrifuge tubes with phenylmethylsulfonyl fluoride (2 mM). Lysates of the labeled cells were prepared as described (10). After centrifugation (50,000 × *g* for 30 min), cell lysates and culture supernatants were precleared by adding to each ml of sample 50 μg of chicken egg albumin and 80 μl of goat-anti-chicken egg albumin (9), incubating the mixtures for 18 hr at 4°C, and removing the precipitates by centrifugation (13,000 × *g* for 30 min). Each 0.5-ml aliquot of these samples was then incubated with 5 μl of anti-idiotype serum, 5 μl of NRS, or 10 μl of rabbit anti-mouse immunoglobulin (RαMIg) for 18 hr at 4°C. The immune complexes were next precipitated by 40 μl of GαRIg. The immunoprecipitates were washed three times with washing buffer (10), suspended in 50 μl of sample buffer [0.06 M Tris·HCl, pH 6.8/10% (vol/vol) glycerol/3% NaDodSO$_4$/5% (vol/vol) 2-mercaptoethanol] and boiled for 2 min. Radioactivity in a 5-μl aliquot was determined (9) and bromophenol blue was added (0.2%) to another 15-μl aliquot of each sample. The samples were subjected to electrophoresis on 13% polyacrylamide slab gels with 0.1% NaDodSO$_4$ and 5.5 M urea for 18 hr

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Abbreviations: Id$^+$, idiotype-positive; NRS, normal rabbit serum; RαMIg, rabbit anti-mouse immunoglobulin; GαRIg, goat anti-rabbit immunoglobulin; V region, variable region of immunoglobulin chains; L, light chain of immunoglobulin; LPS, lipopolysaccharide; P_i/NaCl/EDTA, 10 mM sodium phosphate buffer, pH 7.2/150 mM NaCl/5 mM EDTA.

at 60 V and autoradiographed as described (11).

Affinity Chromatography on DNA-Agarose Columns. Denatured DNA was alkylated with phenylalanine mustard as described (12), with a molar ratio of 1:200 of alkylating agent to nucleotide. The alkylated DNA was conjugated to a hydrophilic amino-substituted agarose (Affigel 102, Bio-Rad) with 1 mg of DNA per ml of gel and 2–5 mg of water-soluble carbodiimide reagent. The DNA-agarose was then washed sequentially with 0.15 M NaCl, 4 M NaCl, 20 mM sodium carbonate at pH 10.5, and P_i/NaCl/EDTA (10 mM sodium phosphate buffer, pH 7.2/150 mM NaCl/5 mM EDTA). The DNA-agarose column was prepared with 0.5 ml in a 1-ml syringe and washed with 5 ml of P_i/NaCl/EDTA followed by 2 ml of elution buffer [20 mM sodium carbonate, pH 10.5/5% (vol/vol) dimethyl sulfoxide] and then with 10 ml of P_i/NaCl/EDTA. The wash was monitored for DNA leaching by measuring *A* at 260 nm. Labeled culture medium or lysate (1 ml) was mixed with EDTA (5 mM) to inhibit DNase and applied to the washed column. The flow-through fractions were collected and the column was washed with 10 ml of P_i/NaCl/EDTA. When the wash reached background levels of radioactivity, the bound antibodies were eluted with 1 ml of elution buffer. Immunoglobulins in the flow-through and eluate fractions were precipitated by 50% saturated ammonium sulfate and dissolved in precipitating buffer (10) for idiotype analysis.

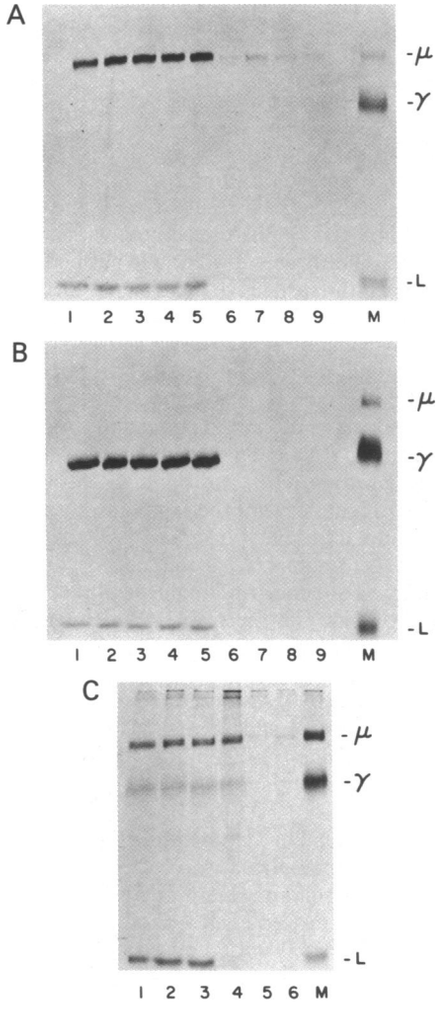

FIG. 1. $NaDodSO_4$/polyacrylamide gel electrophoresis of immunoprecipitates with different antisera and GαRIg. (*A*) [^{35}S]Methionine-labeled H130 hybridoma culture supernatants were immunoprecipitated by anti-H130 (lane 1), NRS (lane 6), anti-H102 (lane 8), or anti-H43 (lane 9). Other lanes contain immunoprecipitates with anti-H130 preincubated with 6 μg of the following immunoglobulins: H102 (lane 2), H43 (lane 3), MOPC-104E (lane 4), MPC-11 (lane 5), or H130 (lane 7). Lane M contains the immunoprecipitate of spleen cell culture supernatant that had reacted with RαMIg. Positions of μ, γ, and light (L) chains were verified by electrophoresis of labeled MOPC-104E and MPC-11. (*B*) H43 hybridoma supernatant immunoprecipitated by anti-H43 (lane 1), NRS (lane 6), anti-H130 (lane 8), or anti-H102 (lane 9). Other lanes contain immunoprecipitates with anti-H43 preincubated with H130 (lane 2), H102 (lane 3), MOPC-104E (lane 4), MPC-11 (lane 5), or H43 (lane 7). (*C*) Biosynthetically labeled MRL/*lpr* spleen cell culture supernatants immunoprecipitated by anti-H130 (lane 1) or anti-H130 preincubated with H43, MOPC-104E, or H130 (lanes 2, 3, and 6, respectively). Lane 5 contains immunoprecipitate with NRS and lane 4 the immunoprecipitate with anti-H130 of MRL/*lpr* spleen cell lysate. Similar results were obtained with anti-H102 and anti-H43 sera, which could be inhibited only by purified H102 and H43 immunoglobulin, respectively (not shown).

RESULTS

***In Vitro* Synthesis of Immunoglobulins with Idiotypic Markers of MRL/*lpr* Anti-DNA Autoantibodies.** Spleen cells from three autoimmune (MRL/*lpr*, MRL/++, and NZB) and five normal (BALB/c, C57BL/6, AKR, SWR, and CBA) strains of mice were incubated with [^{35}S]methionine for 4 hr and the ^{35}S-labeled newly synthesized idiotype-positive (Id^+) immunoglobulins were quantitated by immunoprecipitation and analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis. The specificity of the rabbit anti-idiotype sera was demonstrated by inhibition tests with purified monoclonal antibodies (Fig. 1 and refs. 6 and 7). Cultured spleen cells from the three lupus-prone strains spontaneously synthesized and secreted immunoglobulins with H130, H102, and H43 idiotypes (Table 1). Synthesis of the H130 and H43 idiotypes increased with age, but not to the same extent in the three strains. NZB spleen cells secreted

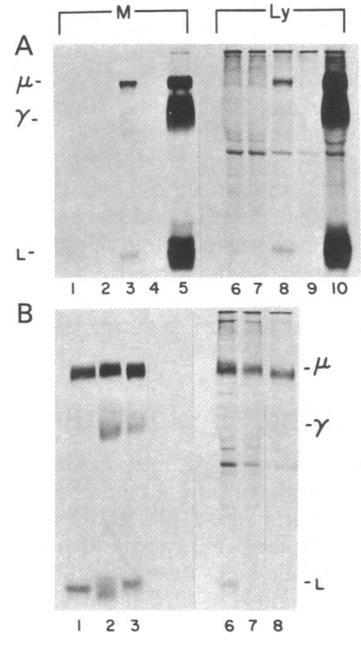

FIG. 2. Gel electrophoresis of immunoprecipitated immunoglobulin synthesized by SWR spleen cells spontaneously (*A*) or upon LPS stimulation (*B*). Immunoglobulins in spleen cell culture supernatant media (M) are shown in lanes 1–5. Lane 1 contains immunoprecipitates of supernatants with anti-H130; lane 2, with anti-H102; lane 3, with anti-H43; lane 4, with NRS; and lane 5, with RαMIg. Lanes 6–10 contain immunoprecipitates of corresponding cell lysates (Ly) with the same antibodies in the same order.

Table 1. Spontaneous synthesis and secretion of Id^+ immunoglobulin by spleen cells in 4-hr culture

Strain	Age,* months	Serospecific cpm† H130	H102	H43	Total Ig
MRL/*lpr*	1	738 ± 161 (0.53)	335 ± 175 (0.24)	1,033 ± 333 (0.74)	140,026 ± 21,184
	4–7	4,953 ± 1,220 (1.05)	837 ± 274 (0.18)	4,058 ± 1,114 (0.86)	473,508 ± 42,003
MRL/++	2–3	251 ± 42 (0.21)	173 ± 33 (0.15)	985 ± 110 (0.83)	118,448 ± 22,176
	12–14	392 ± 95 (0.13)	165 ± 93 (0.05)	1,001 ± 346 (0.32)	308,560 ± 35,272
NZB	1–3	634 ± 187 (0.16)	981 ± 616 (0.25)	4,666 ± 824 (1.18)	397,001 ± 23,150
	5–7	1,102 ± 487 (0.26)	797 ± 203 (0.19)	5,168 ± 773 (1.22)	422,748 ± 88,905
BALB/c	2–3	0	0	49 ± 19	ND
	9	0	0	734 ± 139 (0.81)	90,592 ± 12,565
C57BL/6	1–3	0	0	466 ± 37 (0.54)	86,120 ± 14,142
	7–8	0	0	592 ± 69 (0.66)	89,813 ± 17,960
AKR	1–2	0	0	523 ± 70 (2.07)	25,296 ± 2,182
	8	0	0	1,472 ± 169 (1.62)	91,049 ± 14,360
SWR	1–3	0	0	441 ± 79 (0.55)	80,143 ± 13,539
	6–9	0	0	796 ± 135 (0.78)	102,655 ± 14,260
CBA	2	0	0	65 ± 14	ND

* Three to five mice tested in each group.

† Spontaneous synthesis and secretion is expressed as mean ± SEM of net serospecific cpm in immunoprecipitates obtained from 0.5-ml aliquots of spleen cell culture supernatants (10^7 cells were labeled in 1 ml). In this and subsequent tables the specific cpm for the respective idiotypes and total immunoglobulins synthesized was calculated by subtracting the nonspecifically trapped radioactivity in the NRS + GαRIg immunoprecipitation that was done simultaneously on an equal aliquot of each sample. Radioactivity in the control NRS precipitates was less than 10% of the radioactivity in the experimental immunoprecipitates. Numbers in parentheses represent the amount of idiotype as percent of total immunoglobulin synthesized (mean values). ND, not done.

relatively high levels of the H43 idiotype at both ages tested. The amount of newly synthesized idiotype constituted less than 2% of the total immunoglobulin secreted by the spleen cells (Table 1). $NaDodSO_4$/polyacrylamide gel electrophoresis of immunoprecipitates from spleen cell culture supernatants and lysates demonstrated immunoglobulins with the three idiotypes (not shown). In the MRL/*lpr* strain, the idiotypes were detected in cultures of spleens from 4-week-old mice, not only as IgM but also as IgG. In MRL/++ and NZB mice, by contrast, the IgG switch occurred only in older animals (not shown).

Spleen cells from the five nonautoimmune strains failed to produce detectable amounts of H130 and H102. The H43 idiotype, however, was synthesized by every culture, and it was found only in IgM molecules (Table 1, Fig. 2*A*). We next attempted to induce the synthesis of the H130 and H102 idiotypes by cultured spleen cells from normal mice. When spleen cells from SWR mice were cultured for 5 days with LPS, all three idiotypes were produced in amounts similar to or greater than those in the autoimmune strains (Fig. 2*B* and Table 2). The idiotypes were, moreover, found in both IgM and IgG isotypes (Fig. 2*B*).

We tested the sera of the nonautoimmune strains for the presence of the idiotypes by a competitive radioimmunoassay. The serum samples were tested for their ability to inhibit the binding of ^{125}I-labeled H130, H102, or H43 antibodies to respective anti-idiotype-coated tubes as described (7). The levels of Id^+ antibodies in the sera of these normal mice were below the limit of detection by this assay—i.e., below 100 ng/ml.

Idiotype Synthesis by Fetal Liver B Cells. Fetal liver B lymphocytes also synthesized the MLR/*lpr* anti-DNA antibody idiotypes. In LPS-stimulated cultures, all three idiotypes were synthesized by fetal liver B cells from both the autoimmune strains and the normal SWR strain (Table 3 and Fig. 3). Moreover, the LPS-stimulated SWR fetal liver B cells synthesized the three idiotypes to a similar or even greater extent than the autoimmune strains did (Table 3), and the Id^+ immunoglobulins had both IgM and IgG isotypes (Fig. 3*B*). No idiotype synthesis was detected when the fetal cells were cultured without LPS, even though some immunoglobulin synthesis occurred.

DNA Binding of the Id^+ Immunoglobulins Synthesized by Fetal Liver B Cells. The Id^+ immunoglobulins that LPS-stimulated fetal liver B cells synthesized were characterized further by DNA-agarose affinity chromatography. In efficiency tests of the DNA-agarose columns, 80% of H130 Id^+ immunoglobulins in the culture supernatant of biosynthetically labeled H130 hybridoma cells bound to the column on a single application and only 0.6% bound when the flow-through fraction of the first column was reapplied on a second column (Table 4). In all supernatants of fetal liver cell cultures a small but significant proportion of H130, H102, and H43 Id^+ immunoglobulins (6–20%), including those from SWR mice, bound to DNA (Table 4). The major portion of the three idiotypes, however, did not bind DNA;

Table 2. LPS-stimulated secretion of Id^+ immunoglobulin by spleen cells

Strain	Age, months	Serospecific cpm H130	H102	H43	Total Ig
MRL/*lpr*	2–3	4,227 ± 503	1,537 ± 479	16,663 ± 3,337	806,770 ± 132,359
MRL/++	4–5	774 ± 146	269 ± 41	24,518 ± 2,651	2,316,252 ± 435,305
NZB	2–3	658 ± 56	1,825 ± 896	7,071 ± 1,645	415,672 ± 34,718
SWR	6	8,218 ± 1,996	9,909 ± 1,941	6,978 ± 1,329	471,699 ± 110,753

Spleen cells were cultured with LPS for 5 days, washed, and labeled with [^{35}S]methionine for 4 hr. Id^+ immunoglobulins and total immunoglobulins secreted are expressed as net serospecific cpm in immunoprecipitates of 0.5-ml culture supernatants (10^7 cells were labeled in 1 ml). The values are mean ± SEM of three experiments.

Table 3. Secretion of Id^+ immunoglobulin by fetal liver cells

Strain	Serospecific cpm			
	H130	H102	H43	Total Ig
	Cultured with LPS			
MRL/*lpr*	763 ± 125	486 ± 193	2,792 ± 1,333	117,984 ± 9,498
MRL/++	332 ± 82	ND	1,052 ± 222	50,769 ± 8,292
NZB	1,153 ± 184	467 ± 27	2,147 ± 523	161,183 ± 22,934
SWR	948 ± 198	1,701 ± 279	2,886 ± 834	300,610 ± 88,380
	Cultured without LPS			
MRL/*lpr*	0	0	0	11,976 ± 1,351
MRL/++	0	0	0	10,361 ± 1,487

Fetal liver cells from embryos (15- to 18-day gestation) were carefully prepared to avoid contamination by maternal blood cells (9), cultured for 5 days in the presence or absence of LPS, and then labeled for 4 hr with [^{35}S]methionine. Values represent results of two experiments, expressed as net cpm (mean ± SEM) in immunoprecipitates of 0.5 ml of culture supernatant (10^7 cells were labeled in 1 ml). ND, not done.

i.e., 2- to 10-fold more of the idiotypes appeared in the flow-through than in the eluted fractions. Reapplication of the flow-through to the column did not result in further binding. In some samples the nonbinding idiotypes amounted to 73% of the total idiotype-specific radioactivity applied to the column. Unlike the H130 hybridoma culture fluids, there was a substantial loss of idiotype-specific radioactivity after application of some fetal liver culture supernatants to the columns. That result may have been due to heterogeneity in the Id^+ antibodies secreted by the fetal B cells; some of the antibodies may have had a high affinity that prevented their complete elution from the column. Nevertheless, 60–80% of the idiotype-specific radioactivity could be accounted for in most cases. Id^+ immunoglobulins synthesized by LPS-stimulated MRL/*lpr* spleen cells also showed similar DNA-binding and nonbinding populations (Table 4).

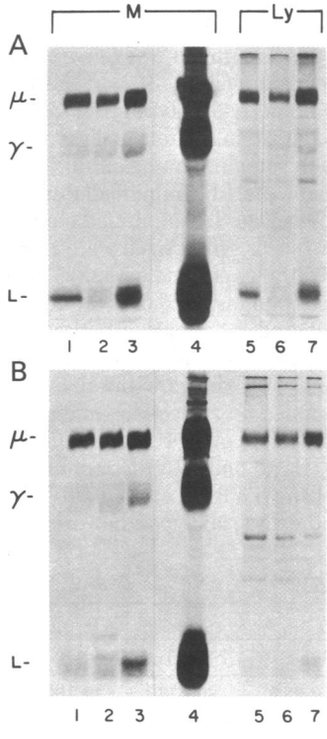

FIG. 3. Gel electrophoresis of immunoglobulins synthesized by LPS-stimulated fetal liver cells of MRL/*lpr* (*A*) and SWR (*B*) mice. Culture supernatants (M, lanes 1–4) and lysates (Ly, lanes 5–7) were immunoprecipitated by anti-H130 (lane 1), anti-H102 (lane 2), anti-H43 (lane 3), RαMIg (lane 4), anti-H130 (lane 5), anti-H102 (lane 6), and anti-H43 (lane 7).

DISCUSSION

The three idiotypes we studied are defined by rabbit antisera against monoclonal MRL/*lpr* anti-DNA autoantibodies, and the idiotype–anti-idiotype reactions are specifically and completely inhibited by DNA and related autoantigens (6). The rabbit antisera thus define structures in the antigen-binding (V) regions of the autoantibodies. The H130 idiotype, or a family of related idiotypes, is a prominent anti-DNA autoantibody idiotype in MRL/*lpr* serum and meets the criteria of a V-region marker of a family of germ-line antibody genes in MRL/*lpr* mice (7). That strain also produces anti-DNA autoantibodies with the H102 and H43 idiotypes but in small amounts (7). All three idiotypes were spontaneously synthesized by cultured spleen cells obtained from three different strains of autoimmune mice. These idiotypes, therefore, are informative probes to determine if B cells from normal mice can express immunoglobulins with V-region markers of the anti-DNA autoantibodies that are produced by mice with a genetic predisposition to SLE.

MRL/*lpr* spleen cells produced large amounts of H130, a result consistent with analyses of MRL/*lpr* serum (7). Substantial amounts of H43 and H102 were also synthesized *in vitro*, in contrast to their low serum levels. Furthermore, only 1% or less of the total immunoglobulin secreted by the cultured spleen cells had the H130 idiotype, even in old MRL/*lpr* mice, whereas in MRL/*lpr* sera the H130 idiotype constitutes up to 28% (mean 10%) of total serum immunoglobulin (7). A similar discrepancy between serum levels and *in vitro* synthesis of a major crossreactive idiotype (CRI) occurs in the anti-*p*-azobenzenearsonate response of A/J mice (13). Moreover, B cells of BALB/c and C3H mice synthesize CRI^+ antibodies in high frequency, although CRI^+ anti-arsonate antibodies cannot be detected in the sera of those strains (14, 15). These findings are similar to our results on the H43 idiotype, which is produced by spleen cells of normal mice but is not detectable in their sera. Differences between *in vitro* and *in vivo* assay systems, immunoglobulin catabolism *in vivo*, and *in vivo* immunoregulatory influences may account for the dissimilar results.

Two of the idiotypes, H130 and H102, were not synthesized spontaneously by cultured spleen cells of five different non-autoimmune strains. The H43 idiotype, however, was synthesized by spleen cells of every normal mouse tested. Upon LPS stimulation of spleen cells from normal SWR mice, synthesis of all three idiotypes was induced at levels equivalent to or even greater than levels found in LPS-stimulated B cells from autoimmune strains. Furthermore, the induced idiotypes were found in both IgM and IgG molecules. Thus, the ability of B cells from genetically disparate strains to express MRL/*lpr* idiotypes indicates that they are members of a conserved family of antibodies. The major idiotype of the anti-phosphocholine

Table 4. Analysis of biosynthetically labeled Id^+ immunoglobulins by chromatography on DNA-agarose columns

Cell culture supernatant	Idiotype-specific cpm								
	Before chromatography			After chromatography					
				Unbound fraction			Bound fraction		
	H130	H102	H43	H130	H102	H43	H130	H102	H43
MRL/*lpr* spleen	3,864	910	10,688	2,581 (67)	523 (57)	4,809 (45)	519 (13)	107 (12)	2,245 (21)
MRL/*lpr* fetal liver	1,980	1,706	3,935	1,440 (73)	650 (38)	1,047 (27)	211 (11)	190 (11)	341 (9)
NZB fetal liver	1,937	896	4,197	1,170 (60)	486 (54)	2,610 (62)	112 (6)	93 (10)	621 (15)
SWR fetal liver	2,292	2,845	4,105	1,546 (67)	1,100 (39)	1,806 (44)	403 (18)	294 (10)	826 (20)

LPS-stimulated adult spleen cells (5×10^6) or fetal liver cells (10^7) were cultured for 4 hr in the presence of [^{35}S]methionine and the supernatants were analyzed for idiotype-specific radioactivity before and after chromatography on DNA-agarose columns. Numbers in parentheses are the percentages of total idiotype-specific radioactivity (cpm before chromatography) that were recovered in the chromatographed fractions; e.g., 13% of H130-specific radioactivity in the MRL/*lpr* spleen cell culture supernatant bound to the DNA column. $NaDodSO_4$/polyacrylamide gel electrophoresis demonstrated that the labeled proteins in each of the idiotype-specific immunoprecipitates consisted only of immunoglobulin molecules (not shown). Efficiency of the chromatography procedure was determined by applying [^{35}S]methionine-labeled H130 hybridoma culture supernatant to a DNA-agarose column and reapplying the unbound fraction to a second column. The H130-specific radioactivity in the material that bound to the two columns and the radioactivity that did not bind after passage on the second column were measured. After a single application of the hybridoma supernatant containing relatively high amounts of H130-specific radioactivity (73,130 cpm), 80% of the H130 antibody (58,780 cpm) bound to the first column and only 0.6% (470 cpm) bound to the second column. About 16% of the monoclonal antibody (11,780 cpm) did not bind even after the second application, probably due to alterations in the DNA binding ability of a small proportion of the immunoglobulin molecules during the procedure.

antibodies of BALB/c mice, T15, is encoded by germ-line genes (16) and is also inducible by LPS stimulation of B cells (17). Polyclonal B cell activation can, therefore, induce expression of apparently unrelated germ-line V genes.

The MRL/*lpr* autoantibody idiotypes were also synthesized by cultured fetal liver B cells of both the autoimmune strains and the normal SWR strain. Idiotype synthesis was detected only when the fetal liver B cells were allowed to mature and proliferate in the presence of LPS. The H130, H102, and H43 idiotypes synthesized by the fetal B cells had DNA binding activity, even when derived from the normal strain. The majority of the Id^+ antibodies, however, did not bind DNA. This result may seem surprising because the three idiotypes were originally defined by monoclonal anti-DNA autoantibodies (6–8). Nevertheless, a similar phenomenon was demonstrated for H130 Id^+ antibodies in MRL/*lpr* serum (7). The H130 idiotypic family seems, therefore, to encompass a group of antibodies, the majority of which do not bind to DNA.

There are other examples of idiotypes that are shared by antibodies with different antigen-binding specificities (18–21). This observation conforms with Jerne's (22) network hypothesis, which postulates regulation of the immune response by anti-idiotypic antibody (Ab2) reacting with idiotypes (Ab1) that are induced by antigenic stimulation. Ab2, according to the hypothesis, also induces and reacts with a nonspecific parallel set of antibodies (Ab3) bearing idiotypes shared with Ab1 but not binding the immunizing antigen. The DNA-binding components of the three idiotype families analyzed here may belong to the parallel set (Ab3) that shares idiotypes with antibodies that are not directed primarily against DNA. Dzierak and Janeway (21) have pointed to the remarkable fact that most inherited dominant idiotypes are directed against bacterial antigens: T15 is anti-phosphocholine and protects mice against pneumococcal infection (23); A5A is anti-streptococcal group A carbohydrate; MOPC 104E is anti-dextran; and UPC-10 is anti-levan (21). The H130-, H102-, and H43-*related* idiotypes that genetically different strains of mice can express may also be directed primarily against certain common pathogens. The binding of those idiotypes with DNA and related antigens could, therefore, represent crossreactions and not unique "anti-self" reactions. Our results suggest that polyclonal B cell stimulation, whether induced by LPS in normal mice or genetically determined in autoimmune mice (9, 24), is the final common pathway to the synthesis of inherited idiotypes with DNA binding activity.

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