Shared Idiotopes and Restricted Immunoglobulin Variable Region Heavy Chain Genes Characterize Murine Autoantibodies of Various Specificities

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Abstract

The study of the Ig variable region heavy chain (V_H) genes used to encode antibodies specific for self-epitopes from murine hybridomas showed that three V_H families are primarily utilized: V_H J558, the largest family, and V_H QPC52 and V_H 7183, the families most proximal to the Ig joining region heavy chain genes. These monoclonal autoantibodies express cross-reactive idiotopes shared by rheumatoid factors and antibodies specific for Sm. The expression of these idiotypes is independent of major histocompatibility complex and Ig constant region heavy chain haplotypes, self-antigen specificity, and even the V_H gene family utilized.

Though the experiments described here are limited to murine autoantibodies, similarities exist between murine and human autoimmune diseases. Studies that aim to investigate the relationship between V_H gene expression and the presence of cross-reactive idiotypes among human autoantibodies should enable us to better understand the mechanisms of autoimmunity and self-tolerance.

Introduction

Autoantibodies of various specificities are not only features of many autoimmune diseases but also occur frequently in chronic infections, aged individuals, and after the use of numerous drugs (1). Thus, a predisposition to autoimmunity has been preserved throughout evolution, and the expression of autoimmunity is surprisingly common.

One striking feature of both human and murine autoantibodies is the incidence of idiotypic crossreactivity (IdX).[1] This has been reported among antibodies to thyroglobulin (2, 3), DNA (4, 5, 6), Sm (7), and acetylcholine receptor (8), as well as with rheumatoid factors (9, 10, 10a). In addition, there is a high degree of IdX among natural murine autoantibodies with multiple specificities (11).

In previous studies, we identified an IdX among murine monoclonal rheumatoid factors (RFs) originating from both normal and autoimmune strains. Moreover, we determined that these monoclonal antibodies (MAbs) are encoded by a limited set of Ig variable region heavy chain (V_H) gene families.

In this communication we report the results of an extended study of a large panel of monoclonal autoantibodies of various specificities. Our analysis indicates that monoclonal autoantibodies of various specificities are encoded by V_H genes from three families and that many of them share IdXs. The expression of this IdX is independent of major histocompatibility complex and Ig constant region heavy chain gene complexes as well as the V_H gene utilized.

Methods

The origin, specificity, and isotypes of the monoclonal autoantibodies used in this study are shown in Table I.

As a control for idiotype (Id) specificity, 16 MAbs specific for influenza viruses (12 for PR8 and 4 for X31) were used. These antibodies are encoded by genes from the V_H 7183 family (19).

Identification of expressed V_H gene families. Briefly, RNA from cytoplasmic lysates prepared from 10^7 cells was fixed to nitrocellulose using a slot-blotting apparatus (20). The filters were baked, hybridized to V_H gene probes, washed, and autoradiographed as described previously (21).

Preparation of V_H gene probes. The V_H-specific fragments representative of the various V_H families were prepared as described previously (22).

Preparation of total RNA and Northern blot analysis. Total RNA was prepared from 3–5 × 10^7 cells using the guanidinium thiocyanate extraction procedure. Northern blotting (used to confirm V_H gene family assignments) was performed by electrophoretically fractionating the RNA on a 1.2% agarose gel (6% formaldehyde) in 40 mM 4-morpholinepropanesulfonic acid, 20 mM NaOAc, and 2 mM EDTA. The gel was blotted without pretreatment onto nitrocellulose using 20× standard saline citrate (3 M NaCl, 0.3 M Na citrate) (22).

Study of idiotypy. The expression of IdX has been studied by competitive inhibition radioimmunoassay. Several polyclonal rabbit anti-Id antibodies were used; their preparation, purification, and specificity have been previously described (3, 23, 10a).

The following anti-Id antibodies were used: anti-LPS 10-1 Id, specific for a BALB/c RF; anti-129-48 Id, an anti-129/Sv RF MAb; anti-Y2 Id, an MRL/*lpr* anti-Sm MAb; anti-62 Id, a BALB/c antithyroglobulin MAb; and anti-PY102 Id, an anti-PR8 influenza virus-specific MAb.

Competitive inhibition radioimmunoassay was carried out as follows. Briefly, microtiter plates were coated overnight at 4°C with 10 μg/ml

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1. *Abbreviations used in this paper:* Id, idiotype; IdX, idiotypic crossreactivity; LPS, lipopolysaccharide; MAb, monoclonal antibody; RF, rheumatoid factor; V_H, Ig variable region heavy chain gene.

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Table I. Origin, Specificity, and Isotypes of Autoantibodies

Origin	Designation and isotypes	Specificity	Reference
6-mo-old 129/Sv "spontaneous"	129-48($\mu\kappa$), 129-78($\mu\kappa$), 129-74($\gamma3\kappa$), 129-102($\mu\kappa$), 129-66($\mu\kappa$), 129-61($\mu\kappa$), 129-76($\mu\kappa$), 129-77($\mu\kappa$)	RF	10a
5-mo-old MRL/*lpr* "spontaneous"	MRL50-8($\mu\kappa$), MRL5-51($\mu\kappa$), MRL22-46($\mu\kappa$), MRL55-26($\mu\kappa$), MRL18-68($\mu\kappa$), MRL55-18($\mu\kappa$)	RF	10a
BALB/c In vitro LPS-stimulated	LPS10-1($\mu\kappa$), LPS7-4($\mu\kappa$), LPS5-4($\mu\kappa$), LPS7-3($\mu\kappa$)	RF	10a
BALB/c Injected with *Y. enterocolitica*	Y19-10($\mu\kappa$), Y19-16($\mu\kappa$), Y43-5($\mu\kappa$)	RF	10a
MRL/*lpr* "spontaneous"	H102($\gamma2a\kappa$), H130($\mu\kappa$), H241($\gamma2a\kappa$), RL1-3	ss DNA	12
BALB/c immunized	HB2($\mu\kappa$)	ds DNA	13
BALB/c immunized	1-15($\gamma1\kappa$), 62Id($\gamma1\kappa$), $B10H_2A_2$($\gamma1\kappa$), APDB6($\gamma1\kappa$)	thyroglobulin	3
CBA/J immunized	$10VA_2$($\mu\kappa$), $10IA_1$($\gamma1\kappa$), $8.4A_3$($\mu\kappa$), $8.ID_2$($\gamma2b\kappa$), $8.IB_1$($\mu\kappa$), $8.4D_1$($\mu\kappa$)	thyroglobulin	14
MRL/*lpr* "spontaneous"	Y2($\gamma2a\kappa$), 2G7($\gamma2a\kappa$), Y12($\gamma2a\kappa$), 6B6($\mu\kappa$)	Sm	7
BALB/c immunized	F8D5($\mu\kappa$)	AcR	
BALB/c immunized	LE4($\gamma1\kappa$)	TSHR	15
NZB "spontaneous"	CP3($\mu\kappa$), CP4($\mu\kappa$)	Br-treated MRBC	16
CBA "spontaneous"	CP5($\mu\kappa$)	Br-treated MRBC	16
DBA/l immunized	A12($\gamma2b\kappa$), B11($\gamma1\kappa$), E8($\gamma1\kappa$), F9($\mu\kappa$), C2($\gamma2b\kappa$), E5($\gamma1\kappa$), F4($\gamma2a\kappa$), F10($\gamma1\kappa$), E7($\gamma1\kappa$), C1($\gamma2a\kappa$), B1	collagen type II	17
BALB/c immunized	HB8($\gamma1\kappa$), HB9($\mu\kappa$)	microfibrils	18
BALB/c immunized	HB10($\mu\kappa$), HB11($\mu\kappa$), HB12($\mu\kappa$)	skin antigens	18

AcR, acetylcholine receptor; Br-MRBC, bromelain-treated mouse red blood cells; ds, double stranded; ss, single stranded; TSHR, thyroid-stimulating hormone receptor.

chromatographically purified anti-Id antibodies. After washing and post-coating with phosphate-buffered saline/bovine serum albumin, the plates were incubated for 2 h at 25° with various dilutions of MAbs (5, 50, and 500 ng/well). After extensive washings, plates were incubated for 2 h at 25° with ^{125}I-labeled Id ligand (50,000 cpm), washed extensively, and radioactivity was counted in a gamma counter. Antibodies yielding at least 40% inhibition were considered IdX-positive.

Results

Identification of V_H gene families

Since we previously determined that mouse monoclonal RFs obtained from various sources were encoded by only certain V_H gene families, it was equally important to determine whether or not other types of autoantibodies also used a restricted set of V_H genes.

Cytoplasmic lysates were prepared from 10^7 hybridoma cells, transferred to nitrocellulose, and assayed by hybridization to a V_H gene-specific probe for each known family (24). With some autoantibodies, the results obtained with the slot-blotting technique were unclear, and, in those cases, the purified RNA was hybridized to the V_H probes using the Northern blotting technique.

Of the 43 autoantibodies that we tested, 17 used a V_H gene from the largest family, V_H J558, 5 used a V_H gene from V_H QPC52, and 20 used a gene from V_H 7183. Only one utilized a V_H gene from the V_H S107 family.

Examples of slot and Northern blotting are illustrated in Figs. 1 and 2. The results of this study, summarized in Table II, indicate that a restricted set of V_H genes encodes murine monoclonal autoantibodies.

Id of monoclonal autoantibodies

Several idiotypic systems previously used to characterize IdX expressed on RF, anti-Sm, and antithyroglobulin autoantibodies were used in this study. All the autoantibodies used were chromatographically purified on a Sepharose 4B rat monoclonal anti-murine kappa antibody column.

^{125}I-Y19-10 anti-LPS10-1 Id antibodies. Y19-10 is a monoclonal RF induced in BALB/c mice by immunization with *Yersinia enterocolitica.* LPS10-1 is a monoclonal RF encoded by a V_H gene from the V_H 7183 family. In this system, a large number of monoclonal autoantibodies with various specificities gave >40% inhibition at a concentration of 10 μg/ml. The results (Fig. 3) show that the inhibition was dose-dependent for the majority of the IdX-positive autoantibodies with the exception of 129-61, LPS7-4, MRL22-46, MRL50-8, $10VA_2$, and CP5, for which an inhibition was not observed with 5 ng of antibody/well.

^{125}I-Y19-10 anti-129-48 Id antibodies. Monoclonal 129-48

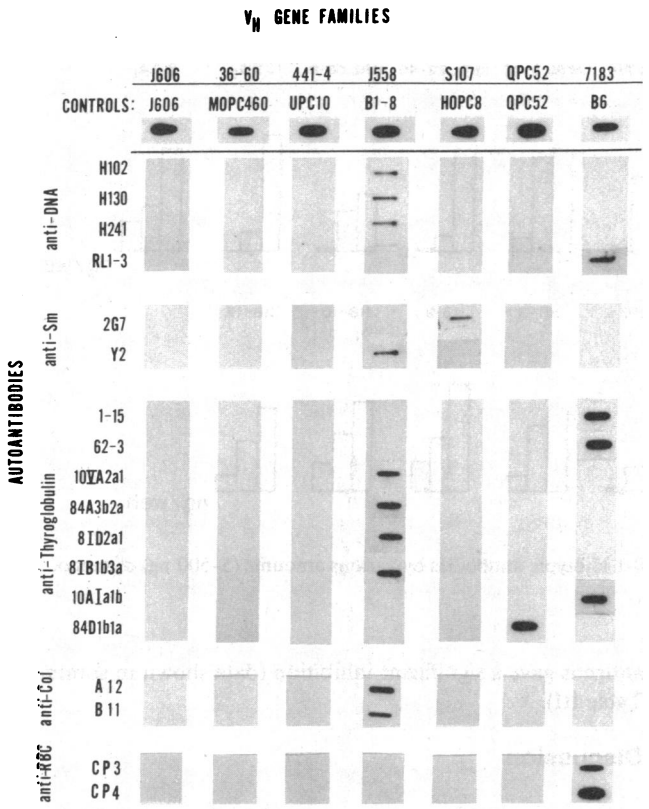

Figure 1. Utilization of V_H genes by monoclonal autoantibodies. Autoradiogram composite of slot blot analysis of cytoplasmic RNA lysates from autoantibodies secreting hybridomas. Nitrocellulose was hybridized to ^{32}P-nick translated V_H gene probes under normal stringency conditions, washed, and autoradiographed. Positive control cell lines from each family are shown at the top of the figure.

is an RF obtained from 129/Sv mice, encoded by a V_H gene closely related to the V_H 37.1 germline gene, a member of the V_H 7183 family (25).

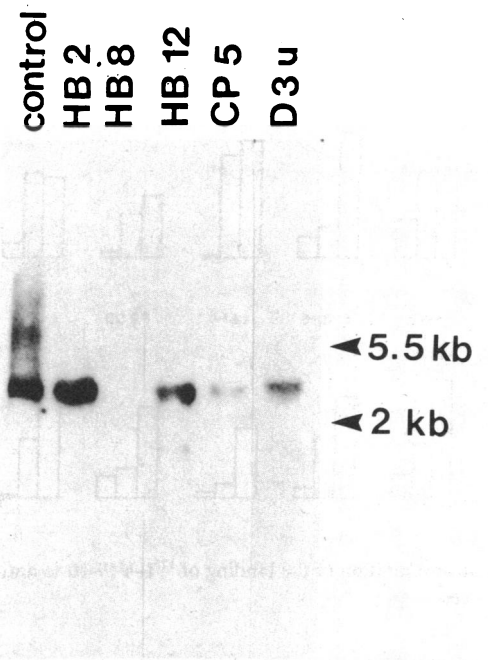

Figure 2. Northern blotting analysis of six hybridomas. 5 μg RNA was electrophoresed through a 1.2% agarose gel (6% formaldehyde), blotted, and hybridized to the ^{32}P-nick translated V_H 129-48 probe.

In this system, an IdX was detected among four monoclonal RFs, two antithyroglobulin antibodies, one anti–collagen type II and two antiskin antigens (Fig. 4).

^{125}I-Y2 anti–Y2 Id antibodies. Monoclonal Y2 is an Sm binding antibody obtained from MRL/*lpr* mice encoded by a V_H gene from the V_H J558 family.

IdX was observed in this system among three anti-Sm antibodies and eight RFs. For some antibodies (LPS10-4, Y43-5, Y19-16, MRL55-26, MRL55-18, and MRL18-68), a strong inhibition was only observed with 1 μg of antibodies/well (Fig. 5).

Table II. Summary Results: Frequency of V_H Gene Families Expressed in Autoantibodies

	Specificity									
V_H family	RF	Thyroglobulin	DNA	Sm	Br-MRBC	TSHR	Collagen type II	Microfibrils	Skin antigens	Frequency
V_H X24										0/43
V_H J606										0/43
V_H 36-60										0/43
V_H J558	MRL50-8	$10VA_2$	H102	Y12, Y2			A12, B11, E8			17/43
	LPS5-4	$8.4A_3$	H130	6B6						
	Y19-10	$8.ID_2$	H241							
	Y19-16	$8.IB_1$								
V_H S107				2G7						1/43
V_H QPC52	MRL5-51	$8.4D_1$					B1	HB8		5/43
	LPS7-3									
V_H 7183	129-48, 129-78,	1-15, 62Id	RL1-3		CP3	LE4	D3μ		HB10 HB12	20/43
	MRL22-46,	$B10H_2A_2$	HB2		CP4					
	LPS10-1, 129-74,	10AI			CP5					
	129-70, 129-78									

Br-MRBC, bromelain-treated mouse red blood cells; TSHR, thyroid-stimulating hormone receptor.

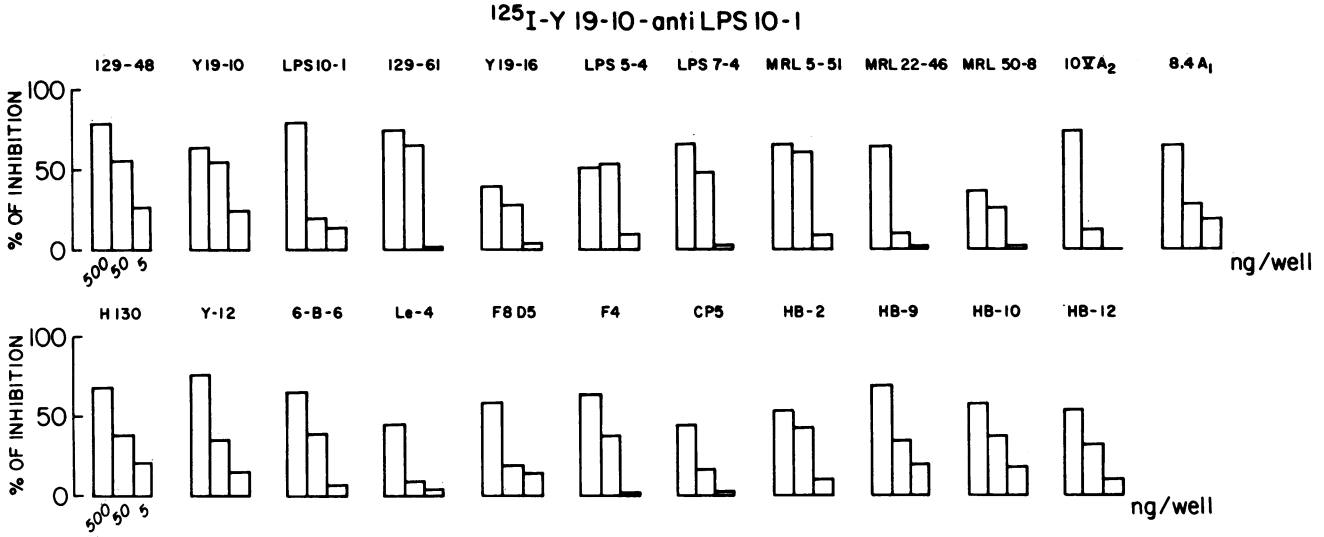

Figure 3. Competitive inhibition of the binding of ^{125}I–Y19-10 to anti–LPS10-1 idiotypic antibodies by various amounts (5–500 ng) of monoclonal autoantibodies.

^{125}I-1-15 anti-62 Id antibody. Monoclonal 1-15 and 62 Id were obtained from BALB/c mice that had been immunized with thyroglobulin. These antibodies are encoded by a V_H gene from the V_H 7183 family. In this system, an IdX was observed on four monoclonal antithyroglobulin antibodies obtained from BALB/c mice but not on other antibodies with different specificities, with the exception of LPS10-1, which gave a weak inhibition (Fig. 6).

It was important to determine if our monoclonal autoantibodies also shared IdX with conventional antibodies specific for foreign antigens. For this purpose we studied the inhibitory activity of 16 antibodies specific for PR8 or X31 influenza viruses in our RF idiotypic systems. These antiviral antibodies were used as controls because they all use a V_H gene from the 7183 family, which is highly represented (20/43) among the autoantibodies.

Only one (VM114) inhibited weakly (at 500 ng/well) the binding of anti–129-48 Id to labeled Y19-10. The other antiinfluenza antibodies showed no inhibitory activity in any system used (data shown only in summary, Table III).

Conversely, we also studied the ability of monoclonal autoantibodies to inhibit an IdX system delineated among antiinfluenza antibodies. In these studies, we utilized labeled VM202 antibody and anti–Py102 Id antibodies. Both (VM202, Py102) are MAb-specific for PR8 influenza virus; they both use a V_H gene from the 7183 family and a V_κ21 light chain (19).

None of our autoantibodies displayed any inhibitory activity in this system, whereas 6 of 16 MAbs specific for influenza virus antigens gave a significant inhibition (data shown in summary, Table III).

Discussion

In an attempt to define more precisely the genetic basis and molecular nature of autoantibodies, we studied the V_H genes and the idiotypy of a panel of 61 monoclonal autoantibodies produced by hybridomas from several mouse strains. Some hybridomas were obtained from mice that spontaneously produce autoantibodies, including RFs obtained from old 129/Sv or MRL/*lpr* mice, anti-DNA and anti-Sm antibodies from MRL/*lpr* mice, and anti–red blood cell antibodies from NZB or CBA mice. Others were obtained from normal immunized animals: e.g., RFs from BALB/c mice injected with *Y. enterocolitica,* antithyroglobulin antibodies from BALB/c or CBA/J mice injected with thyroglobulin, and antibodies against type II collagen from DBA/J mice.

Because our panel of MAbs covers various antigenic specificities, one might expect that all the V_H gene families would be randomly used. However, we found that only a restricted number of V_H gene families are used among these autoantibodies, namely J558 and the most 3′ families: QPC52 and 7183. This restriction is totally independent of combining site specificity and is not related to a spontaneous or induced origin.

It is interesting to note that among these three families the 3′ V_H 7183 gene family is infrequently used by mature B cells, whereas it is frequently used normally in early development (22,

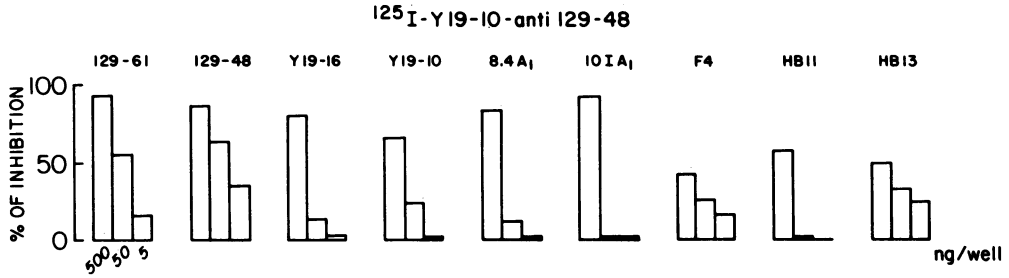

Figure 4. Competitive inhibition of the binding of ^{125}I–Y19-10 to anti-129-48 idiotypic antibodies by various amounts (5–500 ng) of monoclonal autoantibodies.

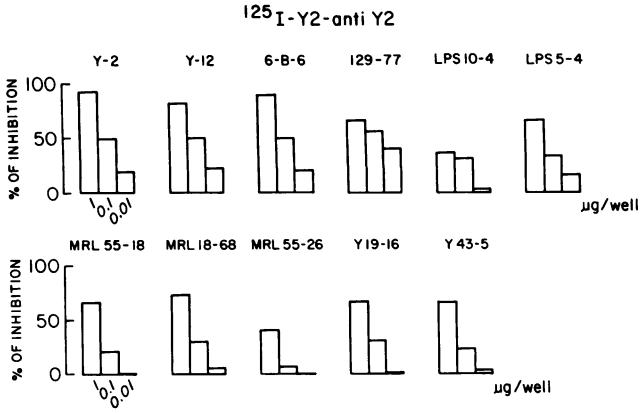

Figure 5. Competitive inhibition of the binding of ^{125}I-Y2 anti–Y2 idiotypic antibodies by various amounts (0.01–1 µg) of monoclonal autoantibodies.

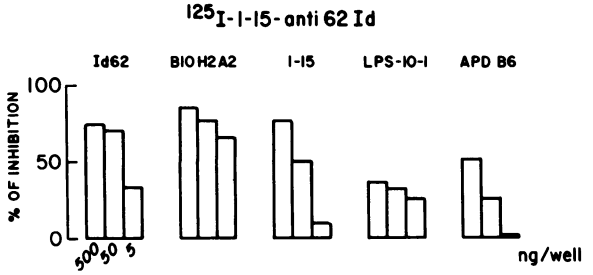

Figure 6. Competitive inhibition of the binding of ^{125}I–1-15 to anti–62 idiotypic antibodies by various amounts (5–500 ng) of monoclonal autoantibodies.

26). Thus, it is possible that these V_H genes used by autoantibodies reflect an immature repertoire of B or pre-B cells. Alternatively, these V_H genes may be representative of a subset of the B cell lineage such as Ly-1 B cells, which secrete a high percentage of IgM autoantibodies (27). This lineage is highly represented in autoimmune strains such as NZB or (NZB × NZW)F_1 mice (28). Moreover, the T1+ B cell subset, a human equivalent of the murine Ly-1 B subset, is significantly increased in patients with rheumatoid arthritis (29).

The autoantibodies studied in this report express IdX despite the fact that they are heterogeneous with respect to combining site and that they utilize different V_H genes.

For example, among our large panel we have identified the presence of IdX originally borne by LPS10-1 and 129-48 RFs and by Y2, a monoclonal anti-Sm antibody. The presence of these IdXs is independent of their specificity, major histocompatibility complex, and Ig constant region heavy chain gene complexes.

Table III. Summary Results: Fraction of Autoantibodies Expressing Crossreactive Idiotypes

	Specificity										
Anti-Id antibodies	RF	Thyroglobulin	DNA	Sm	Br-MRBC	TSHR	Collagen type II	AcR	Microfibrils	Cytoplasmic skin antigens	Influenza virus
Y19-10 anti-LPS10-1-Id	129-48, Y19-10, LPS10-1, 129-61, Y19-16, LPS5-4, LPS7-4, MRL5-51, MRL22-46, MRL50-8	10VA_2 8.4.A_1	H130 HB2	Y12 6B6	CP5	LE4	F4	F8D5	HB9	HB10 HB12	
	10/20*	2/9	2/5	2/4	1/3	1/1	1/10	1/1	1/2	2/3	0/16
Y19-10 anti-129-48 Id	129-48 Y19-10 129-61 Y19-16	8.4A_1 10IA_1					F4			HB10 HB11	VM114
	4/20	2/9	0/5	0/4	0/3	0/1	1/10	0/1	0/2	2/3	1/16
Y2 anti–Y2 Id	129-77, LPS5-4, MRL55-18, LPS10-4, MRL16-68, MRL55-26, Y19-10, Y43-5			Y2 Y12 6B6					ND‡	ND	ND
	8/20	0/9	0/5	3/4	0/3	0/1	0/10	0/1			
1-15 anti–62 Id	LPS10-1	1-15 ADPB6 62ID B10H_2A_2									ND
	1/20	4/9	0/5	0/4	0/3	0/1	0/10	0/1	0/2	0/3	
VM202 anti-PY102 Id	0/20	0/9	0/5	0/4	0/3	0/1	0/10	0/1	ND	ND	6/16

AcR, acetylcholine receptor; Br-MRBC, bromelain-treated mouse red blood cells; TSHR, thyroid-stimulating hormone receptor. * Fraction of antibodies expressing cross-reactive idiotypes. ‡ Not done.

At first glance, the presence of an IdX on antibodies encoded by various V_H gene families is surprising, since it is accepted that IdX are often markers of a particular V_H family, as was shown for antidextran, antinitrophenyl, or antiarsonate antibodies (30).

However, several exceptions to this rule have been reported. For example, antiarsonate antibodies produced by BALB/c mice express IdX despite the fact that they use a V_H gene that does not derive from the V_H 36-65 germline gene (31). In addition, crossreactive Ids were also observed for antibodies of various specificities that were derived from different members of the same family of V_H germline genes (32). Furthermore, our previous molecular data showed that shared IdX can result even if D and JH segments are not identical. It is possible that this kind of IdX is related to a short DNA segment conserved among V_H gene families used by antibodies. In fact, we and others suggest that a conserved framework may be responsible for IdX (10a).

These IdXs shared by autoantibodies of various specificities may be a clue to the genetic and immunoregulatory basis of autoimmunity. It is clear that the cells that produce these autoantibodies are present in normal animals, where they can be induced at times into clonal expansion by hyperimmunization, as in the case of RF. They may also emerge spontaneously during the course of an autoimmune disease, or perhaps even during the aging process.

The precise mechanisms that down-regulate these potentially autoreactive B cell clones are not known, but they probably involve both intracellular (i.e., genetic) and extracellular (i.e., suppression) events. One such intracellular event, based on the findings in this report, would be the utilization of a restricted set of V_H genes. The presence of an unusual B cell subset (e.g., Ly-1 B cells) may be associated or even necessary for this to occur.

Extracellular mechanisms leading to polyclonal B cell activation and autoimmunity may involve failure of T cell suppression, interference with network regulation based on Id recognition, failure of natural killing (33, 34, 35), or some combination of these. If IdX is present on the surface of autoreactive B cells and is recognized by other cells, then its overabundance in autoimmunity may contribute in some way to immunoregulatory failure and disease pathogenesis.

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References

1. N. Talal, editor. 1977. Autoimmunity: Genetic, Immunologic, Virologic and Clinical Aspects. Academic Press, New York.

2. McCoy, J. P., J. H. Michaelson, and P. E. Bigazzi. 1983. Anti-idiotypic immunity and autoimmunity III. Investigations in human autoimmune thyroiditis. *Life Sci.* 32:109–118.

3. Zanetti, M., M. De Baets, and J. Rogers. 1983. High degree of idiotypic cross-reactivity among murine monoclonal antibodies to thyroglobulins. *J. Immunol.* 131:2452–2457.

4. Schwartz, R. S., and D. Stollar. 1985. Origins of anti-DNA autoantibodies. *J. Clin. Invest.* 75:321–327.

5. Halpern, R., A. Davidson, A. Lazo, G. Solomon, R. Lahita, and B. Diamond. 1985. Familial systemic lupus erythematosus. Presence of a cross-reactive idiotype in healthy family members. *J. Clin. Invest.* 76: 731–736.

6. Hahn, B. H., and F. M. Ebling. 1984. A public idiotypic determinant is present on spontaneous cationic IgG antibodies to DNA from mice of unrelated lupus prone strains. *J. Immunol.* 133:3015–3019.

7. Pisetsky, D. S., and E. A. Lerner. 1982. Idiotypic analysis of a monoclonal anti-Sm antibody. *J. Immunol.* 129:1489–1491.

8. Dwyer, D. S., R. J. Bradley, C. K. Urquhart, and J. F. Kearney. 1983. Naturally occurring anti-idiotypic antibodies in myasthenia gravis patients. *Nature (Lond.).* 301:611–614.

9. Andrews, D. W., and J. D. Capra. 1981. Complete amino acid sequence of variable domains from two monoclonal human anti-gammaglobulins of the Wa crossidiotypic group: suggestion that the J segments are involved in the structural correlate of the idiotype. *Proc. Natl. Acad. Sci. USA.* 78:3799–3803.

10. Chen, P. P., S. Fong, D. Normansell, R. A. Houghten, J. G. Karras, J. H. Vaughan, and D. A. Carson. 1984. Delineation of a cross-reactive idiotype on human autoantibodies with antibody against a synthetic peptide. *J. Exp. Med.* 159:1502–1511.

10a. Manheimer-Lory, A., M. Monestier, B. Bellon, F. Alt, and C. Bona. 1986. Fine specificity, idiotypy, and nature of cloned V_H genes of murine monoclonal rheumatoid factor antibodies. *Proc. Natl. Acad. Sci. USA.* In press.

11. Lymberi, P., G. Dighiero, T. Ternynck, and S. Avrameas. 1985. A high incidence of cross-reactive idiotypes among murine natural autoantibodies. *Eur. J. Immunol.* 15:702–707.

12. Rauch, J., E. Murphy, J. B. Roths, B. D. Stollar, and R. S. Schwartz. 1982. A high frequency idiotypic marker of anti-DNA autoantibodies in MRL/lpr mice. *J. Immunol.* 129:236–241.

13. Monier, J. C., J. Brochier, A. Moreira, C. Sault, and B. Roux. 1984. Generation of hybridoma antibodies to double-stranded DNA from non-autoimmune BALB/c strain: studies on anti-idiotype. *Immunol. Lett.* 8:61–68.

14. Rose, N. R., M. A. Accavitti, and M. A. Leon. 1985. Thyroglobulin-specific monoclonal antibodies. *In* Antibodies: Protective, Destructive and Regulatory Role. F. Milgrom, C. J. Abeyounis, and B. Albini, editors. Karger, Basel, Switzerland. 171–178.

15. Cleveland, W. L., N. H. Wasserman, P. Sarangurajan, A. S. Penn, and B. F. Erlanger. 1983. Monoclonal antibodies to the acetylcholine receptor by a normally functioning autoantiidiotypic mechanism. *Nature (Lond.).* 305:56–57.

16. Poncet, P., H. P. Kocher, J. Pages, J. C. Jatou, and A. E. Bussard. 1985. Monoclonal antibodies against mouse red blood cells: a family of structurally restricted molecules. *Mol. Immunol.* 22:541–551.

17. Holmdahl, R., K. Rubin, L. Klareskog, E. Larsson, and H. Wigzell. 1986. Characterization of the antibody response in mice with type II collagen-induced arthritis, using monoclonal anti-type II collagen antibodies. *Arthritis Rheum.* 29:400–410.

18. Dawson, J. F., J. Brochier, D. Schmitt, S. Saeland, and J. Thivolet. 1984. Elastic fibers: histological correlation with orcein and a new monoclonal antibody, HB8. *Br. J. Dermatol.* 110:539–546.

19. Moran, T. M., M. A. Reale, M. Monestier, R. Mayer, J. L. Schulman, and C. A. Bona. 1986. Idiotypy of anti-influenza virus immune response. *Concept. Immunopathol.* 3:233–252.

20. White, B. A., and F. C. Bancroft. 1982. Cytoplasmic dot hybridization. Simple analysis of relative mRNA levels in multiple small cell or tissue samples. *J. Biol. Chem.* 257:8569–8572.

21. Alt, F. W., N. Rosenberg, V. Enea, E. Siden, and D. Baltimore. 1982. Multiple immunoglobulin heavy chain gene transcripts in Abelson murine leukemia virus transformed cell lines. *Mol. Cell. Biol.* 2:386–400.

22. Yancopoulos, G. D., S. V. Desiderio, M. Paskind, D. Kearny, D. Baltimore, and F. W. Alt. 1984. Preferential utilization of the most J_H proximal V_H gene segments in pre B cell lines. *Nature (Lond.).* 311: 727–733.

23. Dang, H., M. Fischback, and N. Talal. 1985. Anti-idiotypic an-

tiserum to monoclonal anti-Sm inhibits the autoantigen-induced proliferative response. *J. Immunol.* 134:3825–3830.

24. Brodeur, P., and R. Riblet. 1984. The immunoglobulin heavy chain variable region (Igh-V) locus in the mouse I. 100 Igh-V genes comprise 7 families of homologous genes. *Eur. J. Immunol.* 14:922–930.

25. Hartman, A. B., and S. Rudikoff. 1984. V_H genes encoding the immune response to β(1-6) galactan: somatic mutation in the IgM molecules. *EMBO (Eur. Mol. Biol. Organ.) J.* 3:3023–3030.

26. Perlmutter, R. M., J. F. Kearney, S. P. Chang, and L. E. Hood. 1985. Developmentally controlled expression of Immunoglobulin V_H genes. *Science (Wash. DC).* 227:1597–1600.

27. Hayakawa, K., R. R. Hardy, M. Honda, L. A. Herzenberg, A. D. Steinberg, and L. A. Herzenberg. 1984. Ly1 B cells: functionally distinct lymphocytes that secrete IgM autoantibodies. *Proc. Natl. Acad. Sci. USA.* 81:2494–2498.

28. Hayakawa, K., R. R. Hardy, D. R. Parks, and L. A. Herzenberg. 1983. The Ly1 B cell subpopulation in normal, immunodefective and autoimmune mice. *J. Exp. Med.* 157:202–218.

29. Plater-Zyberk, C., R. N. Maini, K. Lam, T. D. Kennedy, and G. Janossy. 1985. A rheumatoid arthritis B cell subset expresses a phenotype similar to that in chronic lymphocyte leukemia. *Arthritis Rheum.* 28: 971–976.

30. M. I. Green and A. Nisonoff, editors. 1984. The biology of idiotypes. Plenum Publishing Corp., New York. 19–59.

31. Leo, O., M. Slaoui, J. Marvel, E. C. B. Milner, J. Hiernaux, J. Moser, J. D. Capra, and J. Urbain. 1985. Idiotypic analysis of polyclonal and monoclonal anti-p-Azophenylarsonate antibodies of BABL/c mice expressing the major cross-reactive idiotype of the A/J strain. *J. Immunol.* 134:1734–1740.

32. Victor-Kobrin, C., T. Manser, T. M. Moran, T. Imanishi-Kari, M. Gefter, and C. A. Bona. 1985. Shared idiotopes among antibodies encoded by heavy chain variable region (V_H) gene members of the J558 V_H family as basis for cross-reactive regulation of clones with different antigen specificity. *Proc. Natl. Acad. Sci. USA.* 82:7696–7700.

33. Shoenfeld, Y., and R. S. Schwartz. 1984. Immunologic and genetic factors in autoimmune diseases. *N. Engl. J. Med.* 31:1019–1029.

34. Talal, N. 1978. Autoimmunity and the immunologic network. *Arthritis Rheum.* 21:853–861.

35. Talal, N. 1984. Interferon and natural killer cells in rheumatic diseases. *In* Immunology of Rheumatic Disease. S. Gupta and N. Talal, editors. Plenum Publishing Corp., New York. 141–163.