AUTOANTIBODIES OF VARIOUS SPECIFICITIES ENCODED BY GENES FROM THE V_H J558 FAMILY BIND TO FOREIGN ANTIGENS AND SHARE IDIOTOPES OF ANTIBODIES SPECIFIC FOR SELF AND FOREIGN ANTIGENS

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After the formulation of the clonal selection theory by Burnet (1) and the discovery of idiotypes by Kunkel (2) and Oudin (3), immunology was dominated by the concept that one clone produces one antibody expressing one antigenic marker (idiotype) and recognizing one antigenic determinant (epitope). This paradigm was supported by data suggesting a triple relationship between the sequence of the hypervariable region, specificity of the combining site, and idiotope (4).

This concept was challenged by two unexpected observations: that the same idiotope can be shared by antibodies with various specificities (5), and that a myeloma protein can bind to two different antigens (i.e., MOPC460, which binds to dinitrophenyl hapten and menadione) (6).

Later, we described another category of multispecific antibodies, called epibodies, which bind to idiotopes and to autoantigens (7). Such antibodies can connect the repertoire for foreign antigens and self antigens; Dwyer et al. (8) have shown that certain antidextran antibodies also bind to anti–acetylcholine receptor antibodies via idiotypic interactions.

Recent studies provided numerous examples of multispecific autoantibodies. Thus, it was shown that human monoclonal proteins with rheumatoid factor properties can bind to histones (9), that thymic B lymphocytes from patients with myasthenia gravis secrete mAbs that bind to myosin, α-actinin, and actin (10), and that a high proportion of mAbs obtained from early B cells exhibit multiple self reactivities (11). It was also shown that a human monoclonal macroglobulin with specificity for α(2,8)-linked poly-*N*-acetyl neuraminic acid binds to polynucleotides or denatured DNA (12), or that mAbs obtained from mice immunized

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with phenylarsonate (13) or *Streptococcus pyogenes* M type 5 (14) bind to DNA, cytoskeleton, myosin, or keratin, respectively.

In this study we addressed two questions: Do "bona fide" autoantibodies obtained from autoimmune mice or from animals immunized with autoantigens in complete Freund's adjuvant (CFA) bind to foreign antigens? Do autoantibodies with a given specificity share idiotopes with autoantibodies of different specificities and with antibodies specific for foreign antigens?

We found that it is important to address these questions because it is not clearly known if the expression of self-reactive clones is driven by autoantigens or exogenous antigens.

Our study was carried out on antibodies encoded by genes from the V_H J558 family selected from our panel of 63 mAbs (15).

The choice of this group of autoantibodies was made for several reasons: (*a*) We possess a large panel of foreign antigens known to bind to antibodies encoded by genes from the V_H J558 family. (*b*) Victor-Kobrin et al. (16) have previously shown that there is a high connectivity among antibodies with various specificities encoded by V_H genes from this family. (*c*) We possess multiple crossreactive idiotypic systems expressed on V_H J558$^+$ antibodies specific for foreign and self-reactive antigens.

The results presented in this communication show that 9 of 20 autoantibodies also bind to foreign antigens and that they share idiotopes with antibodies specific for autoantigens or foreign antigens.

Materials and Methods

Antigens. The panel of antigens used in this study is illustrated in Table I. They are known to bind to murine antibodies encoded by V genes derived from the V_H J558 family (17).

Cardiolipin, thyroglobulin and hen eggwhite lysozyme were purchased from Sigma Chemical Co., St. Louis, MO. Phenylarsonate and nitrophenyl acetate conjugates were a gift from Drs. M. Gefter and T. Imanishi (Massachusetts Institute of Technology, Cambridge, Massachusetts), and dextran antigens were from Dr. E. Kabat (Columbia University, New York). Synthetic antigens were obtained according to a previously described technique (18), and the lipopolysaccharides of our own collection were extracted from various gram-negative bacteria by the method of Westphal et al. (19).

Monoclonal Antibodies

The origin, specificity, and isotypes of the mAbs used in this study are shown in Table II. All of these autoantibodies have been previously identified as using a V_H gene derived from V_H J558 family (15).

Binding to Antigens

The binding of the V_H J558$^+$ autoantibodies to various antigens was determined by radioimmunoassay (RIA). Microtiter plates were coated overnight at 4°C with 10 μg/ml antigen. After washing and postcoating with BSA, the plates were incubated for 2 h at 20°C with various concentrations of antibodies. After extensive washings, plates were incubated for 2 h at 20°C with ^{125}I-labeled rat anti-mouse κ mAb (50,000 cpm/well), washed extensively, and the radioactivity was counted in a gamma counter.

Specificity of the binding was studied by using a competitive inhibition RIA. This technique was carried out in two steps: (*a*) in liquid phase, 0.5 μg of antibody were incubated with various amounts of antigens (15 and 150 ng) for 2 h at room temperature in microtubes coated previously with BSA, and (*b*) 50 μl of this mixture was transferred to microplates coated with antigens as described above.

TABLE I
Foreign Antigens Used in This Study

Source or type	Antigens
α1–3, 1–6 Dextran	B1948L, B1355S, B512, B1254S, B1510S, B1375, B1424, B1141, B1425, B1355L, B1498S, B742S, B1399, B1501L, B742L, B1142, B1299SB, B1255
Influenza virus	PR8(H1N1), X31(H3N2), B/Lee, A Singapore (H1,N1)
Synthetic peptides	Poly(Glu^{24}, Lys^{16}, Ala^{60}) (GLA^{60}) Poly(Glu^{54}, Lys^{37}, Phe^{9}) (GLφ) Poly(Glu^{50}, Tyr^{50}) (GT) Poly(Glu^{60}, Phe^{40}) (Gφ) Poly(Glu^{60}, Lys^{40}) (GL) Poly(Glu^{60}, Ala^{30}, Tyr^{10}) (GAT)
Lypopolysaccharides	*Proteus morganii* 4B III *Escherichia coli* 0113 *Proteus vulgaris* 5CIII *Providencia stuartii* 12A X *Salmonella minnesota* R 595 *Klebsiella oxytoca* 9B IV *S. tranaora* *Proteus mirabilis* 3A III *Bacteroides fragilis* *S. thompson* S-PP-385 *Pseudomonas aeruginosa* *Klebsiella pneumoniae* 7A IV *S. anatum* S-PP-385 *E. coli* 0111 27C XI *S. newington* *Neisseria lactamica* 26A XIV *Enterobacter cloacae* 10A VII *Shigella dysentariae* 20C VIII *Serratia marcescens* 6 AV *Pseudomonas fluorescens* 2C II *E. coli* K235
Haptens	Nitrophenyl acetate–chicken IgG, phenylarsonate-BSA
Protein antigens	Hen lysozyme

Affinity Measurement

Affinity measurements were carried out as described by Friguet et al. (20). Briefly, in a preliminary experiment, each antibody at various concentrations was incubated for different lengths of time in microtiter plates coated with the antigen. The content of each well was transferred into another coated well and incubated for the same time. In the two series of wells, the bound antibody was revealed using ^{125}I-labelled anti-κ as described before. This procedure was performed to determine that no readjustment of the equilibrium in the liquid phase would occur during the experiment aimed to measure affinity. We considered a time period satisfactory when the binding in the second set of wells was not less than 85% of the binding observed with the first set. The affinity was measured using an RIA similar to the one described previously. Antibody at a known concentration was incubated overnight with various amounts of antigen in PBS-BSA. The antibody-

TABLE II
Characteristics of Autoantibodies Used in This Study

Designation	Origin	Specificity	Isotype	V_H	Reference
10VA2	CBA/J mice immunized with TG	Thyroglobulin	μk	J558	42
84 A3			μk	J558	
8I B1			μk	J558	
8I D2			γ2bk	J558	
H102	MRL/*lpr* spontaneous	DNA	γ2ak	J558	43
H130			μk	J558	
H241			γ2ak	J558	
E8	DBA/1 immunized with type II collagen	Type II collagen	γ1k	J558	44
A12			γ2bk	J558	
Y2	MRL/*lpr* spontaneous	Sm	γ2ak	J558	23
Y12			γ2ak	J558	
6B6			μk	J558	
15–32	SJL immunized with MBP	Myelin basic protein	γ2ak	J558	45
S2-9-2	Motheaten spontaneous		μk	J558	46
UN59-9			μk	J558	
6-19-23	MRL/*lpr* spontaneous		γ3k	J558	46
MRL50-8			μk	J558	22
Y19-10	BALB/c immunized with *Y. enterocolitica*	Rheumatoid factor	μk	J558	22
Y19-16					
LPS5-4	BALB/c in vitro LPS stimulation		μk	J558	
			μk	J558	

antigen mixture was then transferred into antigen-coated wells and incubated for the time determined in the preliminary experiment. The binding was revealed by ^{125}I–anti-κ antibody. The calculations for the affinity determination were performed according to Friguet et al. (20) and the K_d is expressed in grams per milliliter instead of molar because of the nature and the source of the antigens used (varying molecular weights and heterogeneity of polymers). The same expression of K_d was used by Sharon et al. (21) to measure the affinity of antibodies specific for dextran, a complex natural antigen.

Study of Idiotypy

Two groups of idiotypic systems were used in this study, defining crossreactive idiotypes (IdX)[1] of autoantibodies and of antibodies specific for foreign antigens. These idiotypes are expressed on antibodies encoded by V_H J558 family–derived V_H genes.

Idiotypes of Autoantibodies. Y19-10 anti-LPS 10-1 defines a crossreactive idiotype recognized by polyclonal rabbit antibodies produced by immunization with LPS 10-1, which is a BALB/c mAb exhibiting rheumatoid factor (RF) activity (22). These rabbit anti-Id antibodies recognize an IdX on Y19-10, a monoclonal RF obtained from a BALB/c mouse immunized with *Yersinia enterocolitica* (22).

Anti-Y2Id is a rabbit anti-Id antibody raised against Y2, an anti-Sm mAb obtained from MRL/*lpr* mouse (23).

[1] *Abbreviations used in this paper:* Ars, *p*-azophenyl arsonate; IdX, crossreactive idiotype; RF, rheumatoid factor.

H130 is a monoclonal anti-DNA antibody obtained from MRL/*lpr* mouse and 108, an anti-Id mAb against H130 (24).

Y19-10, LPS10-1, Y2, and H130 use genes derived from the V_H J558 family (15).

Idiotypes of Antibodies Specific for Foreign Antigens. J558-CD3-2 defines J558 IdX expressed on a majority of α-1-3-dextran antibodies. CD3-2 is a previously described monoclonal anti-J558 IdX antibody, kindly donated by Dr. J. Kearney (Univ. of Alabama, Birmingham, AL) (25).

G5 is an mAb specific for the GAT terpolymer. HP20 is an mAb recognizing a GAT-crossreactive idiotype (26). Both reagents are a gift from Dr. M. Fougereau (Centre Inserm, Marseille-Luminy, France).

The IdX of antiarsonate (anti-Ars) antibodies was defined by 36-65-AD8, mAbs previously described (27). 36-65, an anti-Ars mAb is a gift from Dr. M. Gefter (MIT, Cambridge, MA), and AD8 is from Dr. G. Lewis (University of Maryland, Baltimore, MD).

PY211 is an mAb specific for PR8 influenza virus hemagglutinin and 63.4, a syngeneic mAb recognizing a crossreactive idiotype on Py211 (28).

PY206 is an mAb specific for X31 influenza virus hemagglutinin and SN3.9A, a syngeneic mAb recognizing a crossreactive idiotype on PR8- and X31-specific antibodies (28).

IDA23 is an anti-A48-Id mAb and AIDA23, a syngeneic anti(anti-Id) mAb. These reagents were kindly donated by P. Legrain (Pasteur Institute, Paris, France).

J558, G5, 36-65, PY211, PY206, and IDA23 are mAbs encoded by V_H genes from the V_H J558 family (26, 27, 28, 29).

Presence of crossreactive idiotypes was determined by a competitive inhibition RIA. Briefly, microtiter plates were coated overnight at 4°C with chromatographically purified anti-Id antibodies (10 μg/ml) in carbonate buffer, pH 9.2. After washing and postcoating with PBS-BSA, the plates were incubated with the antibodies to be tested (10 μg/ml in PBS-BSA). After 2 h at 20°C, the plates were washed and incubated with 50,000 cpm of the corresponding idiotype labelled with ^{125}I. After extensive washing, radioactivity was counted using a gamma counter.

The specificity of the idiotypic systems shared by $J558^+$ antibodies directed against foreign antigens was determined by using mAbs, prototypes of each of the seven major murine V_H families.

Results

Binding to Foreign Antigens. The binding activities of 20 V_H $J558^+$ autoantibodies with various specificities were tested against both the corresponding autoantigen and all foreign antigens, included our panel (Table I). 9 of 20 autoantibodies studied exhibited various degrees of binding to foreign antigens. The data presented in Fig. 1 show a dose effect relationship of binding for 15-32, H102, H130, and UN59-9, whereas a binding by high doses of antibody (3 and 10 μg/ml) was observed with the other antibodies. The competitive inhibition assay was carried out to determine whether or not the binding to auto- and foreign antigens is an intrinsic property of the antibody-combining site. The data presented in Table III show that the binding of autoantibody to the corresponding autoantigen was antigen-inhibitable (excepting 15-32, for which the binding to GLϕ was not inhibited by MBP). Similarly, the binding to foreign antigen by autoantibody was inhibited by the corresponding autoantigen as well as by the foreign antigen. We also examined the binding to phosphocholine, levan, inulin, and TNP. These antigens are known to bind to antibodies encoded by V genes from the V_H S107, V_HX24, V_H J606, and V_H 36-60 families, respectively (17). No binding to these antigens by our V_H $J558^+$ autoantibodies that bound to foreign antigens was observed (data not shown).

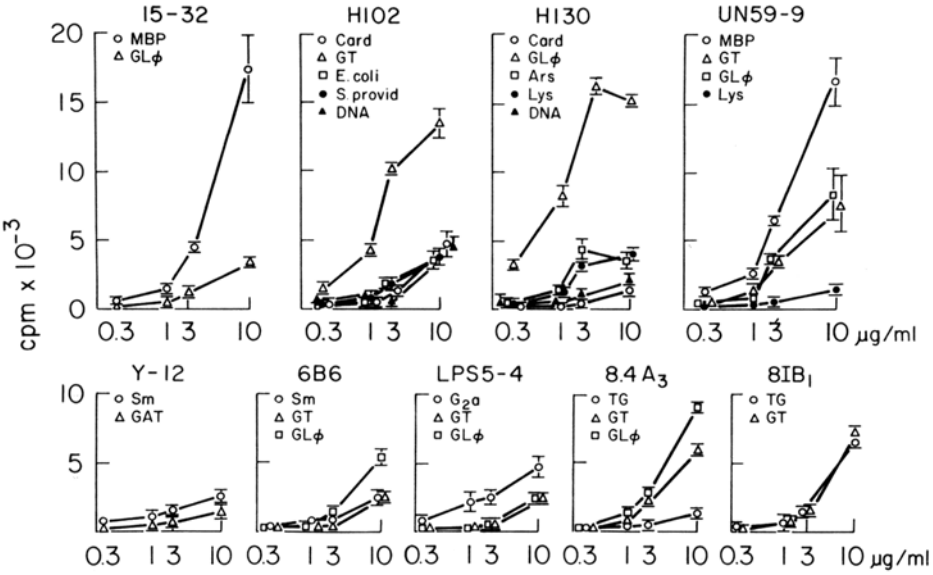

FIGURE 1. Binding of autoantibodies to auto- and foreign antigens assayed by RIA. Microtiter plates coated with antigen were incubated 2 h with 0.3, 1, 3, or 10 μg/ml of antibody in PBS-BSA. After washing, the binding was revealed using ^{125}I-labeled monoclonal anti-κ. Abbreviations as in Table I.

We determined the affinity of binding of the 9 out of 20 antibodies that showed crossreactivity with foreign antigens. The data presented in Table IV show that the K_d is usually lower for autoantigens than for foreign antigens, with the exception of Sm-specific autoantibodies (6B6 and Y12). It should be mentioned that because of the particularly complex nature of the antigen used, the K_d were expressed as grams per milliliter, as was done by Sharon et al. (21) with antidextran antibodies.

We also investigated the binding to GT, GAT, and GLϕ of a panel of 30 autoantibodies encoded by V genes from V_HQP52 and V_H7183 families (Table V). We did not observe significant binding of these antibodies, with the exception of a monoclonal anti-DNA antibody (HB2), which showed binding to GT.

Study of Expression of Crossreactive Idiotypes. In further experiments, we examined our V_H J558$^+$ autoantibodies for the presence of IdX originally borne by autoantibodies and by antibodies specific for foreign antigens and encoded by V_H J558 genes. There are three reasons for our study: First, we have shown the existence of a high idiotypic connectivity among autoantibodies of various specificities (18). Second, we found that 9 of our 20 autoantibodies bind to foreign epitopes and, since IdX are markers of germline genes (30), the presence of IdX common to autoantibodies and antibodies specific for foreign antigens may indicate a common germline origin. Third, the presence of IdX among autoantibodies and antibodies specific for foreign antigen can shed light on the activation mechanism of self-reactive clones, since it is known that anti-Id antibodies can influence the expansion of autoreactive clones (reviewed in 31).

The summary results of these studies are shown on Table VI and were determined by RIA in a dose-response manner: 15 of 20 antibodies express the

TABLE III

Inhibition of Binding of Autoantibodies to Self Antigens by Foreign Antigens

mAb (10 μg/ml)	Binding to plates coated with:	Percent inhibition of binding with:*									
		MBP	GLϕ	GT	Lysozyme	Sm	TG	GZa	DNA	Ars	*E. coli*	*S. providencia*
15-32	MBP	6.3	61.7									
	GLϕ	0	72									
UN59-9	MBP	53.5	83.6	86.5	54.6							
	GT	77		88.1								
	GLϕ	62.5	70.8									
	Lysozyme	55			60.6							
6B6	Sm		58.2	50.9		55.3						
	GT			82.6		73.3						
	GLϕ		86.1			80.7						
Y-12	Sm		40.6			42.4						
	GAT		76.6			79						
81B1	TG		84.8				76.3					
	GT		88.2				74.4					
84A3	TG		80.3	80.6			62.3					
	GT			81.9			52.5					
	GLϕ		91.4				66.7					
LPS5-4	G2a		53.1	46.5				54.3				
	GT		66	70.1				59.6				
	GLϕ		68.6	71.2				65.5				
H130	DNA		78.4	83.8	60				50.9	67.6		
	Cardiolipin		79.7	73.6	90.6				70	94.2		
	GLϕ		52.9						20			
	GT			83					86.3			
	Lysozyme				55.3				58.3			
	Ars								84	83.5		
H102	DNA			77.2					75.8		65	66.5
	Cardiolipin			44					86		91.7	74.5
	GT			81.3					61			
	E. coli								58.3		53.7	
	S. providencia								58.7			70.8

* Antigens used at 15 ng/well.

idiotype of Y19-10, a monoclonal RF; 6 of 20 express the Y2 idiotype, a monoclonal anti-Sm antibody; and 6 of 20 express an idiotype originally borne by H130, an anti-DNA mAb. An example of dose-dependent inhibition is shown in Fig. 2, where various amounts of 10VA2 have been used to inhibit the binding of labelled H130 to rabbit anti-H130 Id antibodies. A similar competitive RIA was used to study the presence of IdX originally expressed on V_HJ558$^+$ antibodies specific for foreign antigens.

Table VII shows the specificity of our idiotypic system, in which a strong inhibition of binding was observed with the corresponding idiotype. Seven purified antibody proteins, prototypes of the major murine V_H families, did not cause inhibition, except J558 in the J558-CD3-2 idiotype system. The data presented in Table VIII summarize the results of this study, which show that 3 of 30 autoantibodies share the J558-IdX; 3 of 20 share the PY211 IdX, an anti–

TABLE IV
Dissociation Constants of mAbs with Self and Foreign Antigens

Antibody	Antigen	K_d (g/ml)
15-32	MBP	5.2×10^{-5}
UN59-9	MBP	1.2×10^{-4}
	GT	9.4×10^{-5}
	GLϕ	1.0×10^{-5}
	Lysozyme	8.9×10^{-5}
6B6	Sm	6.6×10^{-5}
	GT	7.6×10^{-5}
	GLϕ	8.0×10^{-5}
Y12	Sm	1.8×10^{-4}
	GAT	6.5×10^{-5}
81D1	TG	7.8×10^{-5}
	GT	7.8×10^{-5}
84A3	TG	1.2×10^{-4}
	GT	7.0×10^{-5}
	GLϕ	7.8×10^{-5}
H130	DNA	1.2×10^{-4}
	GLϕ	6.6×10^{-5}
	GT	1.2×10^{-4}
	Lysozyme	1.6×10^{-4}
	Ars	5.3×10^{-4}
H102	DNA	1.4×10^{-6}
	GT	5.3×10^{-5}
	E. coli	5.2×10^{-5}
	S. providencia	1.6×10^{-4}
LPS5-4	γ2a	1.2×10^{-4}
	GT	4.5×10^{-5}
	GLϕ	8.0×10^{-5}

influenza virus hemagglutinin antibody; 3 of 20 share an IdX present on G5, a GAT-specific antibody; and 3 of 20 share an IdX on 36-65, an anti-Ars antibody.

It should be mentioned that none of the autoantibodies encoded by V_HQP52 and V_H 7183 family genes (Table V) expressed the IdX originally borne by V_H J558$^+$ antibodies directed against foreign antigens (data not shown).

Discussion

In this communication, we present results showing that autoantibodies obtained from animals prone to develop autoimmune disease, or animals actively immunized with autoantigen bind to foreign antigens and share idiotypes with autoantibodies of various specificities, as well as with antibodies specific for foreign antigens. In our study, we only used autoantibodies encoded by genes

TABLE V
Autoantibodies Encoded by Genes Derived from 3′ V_H Families

V_H families	Designation	Specificity
QPC52	MRL5-51, LPS 7-3	RF
	84D1B1	TG
	HB8	Skin antigens
7183	B36, Z26, M93, M16	Sm
	Z317, Z121, Z41, Z49, HB2	DNA
	M88, B57, B61, B56, MRL22-46 LPS10-4, 129-48, 129-78	RF
	CP3, CP4	RBC
	Id62, 1-15, B10 H_2 AD_2	TG
	LE4	TSH receptor
	C2	Collagen type II
	HB10, HB12	Skin antigens

TABLE VI
Autoantibodies with Various Specificities Sharing Crossreactive Idiotypes with Autoantibodies Encoded by V_H J558 Family Genes

Idiotypic system	Specificity of idiotype	Autoantibodies sharing crossreactive idiotypes	Frequency
Y19-10–anti-LPS10-1	RF	Y19-10(RF), LPS5-4(RF), Y19-16(RF), MRL50-8(RF), Y12(Sm), 6B6(Sm), H102(DNA), H130(DNA), 15-32(MBP), 84A3(TG), 10VA2(TG), 81 B1(TG), 81D2(TG), UN59-9(MBP), S2-9-2(MBP)	15/20
Y2–anti-Y2	Sm	Y-2(Sm), Y-12(Sm), 6B6(Sm), LPS5-4(RF), Y19-16(RF), 6-19-23(RF)	6/20
H130–anti-H130	DNA	H130(DNA), H102(DNA), H241(DNA), 10VA2(TG), 81 B1(TG), 6B6(Sm)	6/20

derived from the V_H J558 family, since we possess a large panel of antigens known to bind to antibodies encoded by the same V_H gene family. The binding studies to foreign antigens show that 9 of 20 antibodies bind with varying degrees to foreign antigens, and particularly to synthetic antigens such as GT and GLϕ. Two anti-DNA antibodies showed significant binding to other antigens: H130 to lysozyme and Ars, and H102 to *E. coli* and *S. providenciae.*

Competitive inhibition experiments demonstrated the specificity of this binding, and affinity measurements showed values commonly found in antigen/antibody interactions, even though the particular nature of some antigens and their lack of purity did not allow us to draw definite conclusions from this affinity measurement.

However, the K_d of "bona fide" antibodies directed against foreign antigens was usually lower than the K_d of multispecific antibodies. While the K_d of various autoantibodies binding to GT varies between 1.2×10^{-4} and 9.4×10^{-5}, G5, a

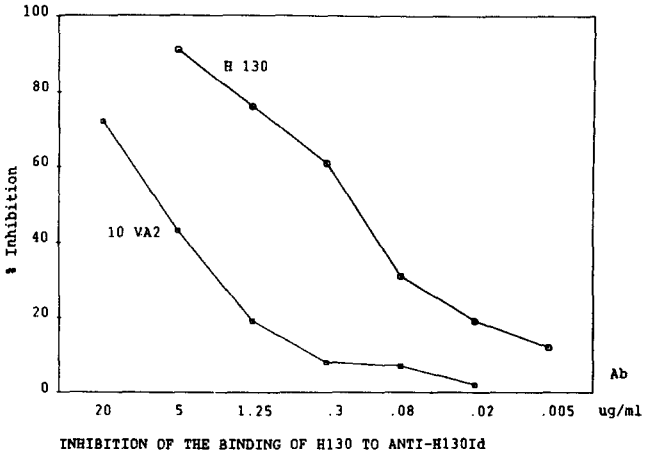

FIGURE 2. Example of competitive inhibition RIA. Microtiter plates were coated with anti-H130-Id (10 μg/ml) and incubated 2 h with various amounts of mAb in PBS-BSA. After washing, ^{125}I-labelled H130 was added. The results are expressed in percent inhibition of binding of ^{125}I-H130 to anti-H130-Id. H130 is an anti-DNA mAb and 10VA2 is an anti-TG mAb sharing IdX with H130.

monoclonal anti-GT antibody, had a K_d of 1.4×10^{-7}. The K_d of H130 to arsonate (5.3×10^{-4}) or to lysozyme (1.6×10^{-4}) was also higher than the K_d of 36-65 (4.3×10^{-6}), an anti-Ars mAb or than the K_d of four antilysozyme mAbs (1C8, 1.1×10^{-7}; 17, 5.7×10^{-8}; 2E5, 3.1×10^{-8}; and 2F4, 7.2×10^{-8}). The higher affinity of "bona fide" antibodies was expected because these clones have been expanded in vivo and selected in vitro by the corresponding antigen. However, small differences in the affinity of autoreactive clones for foreign antigens compared with the clones expanded by the same antigens suggest the possibility of the activation of autoreactive clones by foreign antigens.

Our data are in agreement with other findings that demonstrated that mAbs obtained from 6-d-old BALB/c mice exhibit a high frequency of self-reactivity, and that eight of these antibodies bound to TNP and self antigens such as actin, tubulin, and myosin (11). Guilbert et al. (32) have also shown that mice hyperimmunized with various antigens in CFA produced multispecific antibodies directed against foreign and autoantigens. Naparstek et al. (33) have shown that the unmutated V_H Id^{CR} gene, which encodes most of anti-Ars antibodies in A/J mice, also encodes antibodies binding to DNA and cytoskeletal proteins.

This crossreactivity for self and foreign antigens can be important in the breaking of self-tolerance. Patients with acute rheumatic fever have antibodies reacting with heart tissue (34), brain (35), and skeletal muscles (36). Because of the polyclonal nature of these antibodies, immunochemical studies aimed at defining their precise specificity have been difficult. However, these observations have been confirmed by studies using mAbs. Thus, mAb obtained from a BALB/c mouse and directed against *S. pyogenes* M type 5 also reacted with muscle proteins (14).

Taken collectively, these data suggest that autoreactive clones can be activated either directly by foreign antigens sharing epitopes with autoantigens, or by the intrinsic ability of self-reactive clones to bind both foreign and autoantigens. Both mechanisms can be involved in the pathogenesis of autoimmune disease.

The most striking observation in our study is the extensive idiotype crossreactivity among V_H $J558^+$ autoantibodies with other autoantibodies and antibodies specific for foreign antigens. Idiotypes originally borne by RF, anti-DNA, and

TABLE VII

Specificity of Anti-Id mAbs Recognizing Crossreactive Idiotype on Antibodies Encoded by V_H J558 Gene Family as Assessed by Competitive RIA

Idiotypic systems	Antigen specificity of idiotype	Binding of Id to corresponding antiidiotype*	Binding of labeled idiotype in presence of 500 ng of:							
			Corresponding idiotype	UPC 10 (X24)‡	J606 (J606)	MOPC460 (36-60)	J558 (J558)	TEPC15 (S107)	IDA 15 (QPC 52)	PY102 (7183)
IDA23-AIDA23.2	IDA23 Id	1,739 ± 169	532 ± 28 (IDA23)§	1,987 ± 25	1,790 ± 121	1,953 ± 111	1,848 ± 43	1,962 ± 42	1,764 ± 63	1,686 ± 64
J558-CD3.2	α1-3-Dextran	4,291 ± 649	1,018 ± 71 (MOPC 104)	2,824 ± 197	3,284 ± 668	2,593 ± 65	840 ± 82	3,600 ± 24	3,353 ± 163	3,103 ± 73
PY211-63-4	HA of PR8	18,545 ± 693	6,900 ± 776 (PY211)	18,384 ± 211	15,389 ± 565	17,888 ± 661	11,957 ± 843	16,813 ± 3,574	18,403 ± 255	17,314 ± 661
PY206-SN3A	HA of X31	12,376 ± 381	1,895 ± 219 (PY206)	ND	ND	ND	ND	ND	ND	ND
G5-HP20	GAT	10,923 ± 3,717	2,700 ± 191 (G5)	11,116 ± 718	9,541 ± 1,714	11,116 ± 718	11,275 ± 909	9,752 ± 724	9,823 ± 845	10,021 ± 414
36-65-AD8	Ars	17,808 ± 202	3,837 ± 246 (36-65)	17,909 ± 568	17,100 ± 751	16,866 ± 656	14,273 ± 368	16,738 ± 3,583	17,551 ± 384	16,581 ± 78

* Average cpm of triplicates ± SD.

‡ V_H family.

§ Corresponding idiotype used in competitive inhibition RIA.

TABLE VIII

Autoantibodies Sharing Crossreactive Idiotypes Expressed on Antibodies Specific for Foreign Antigens and Encoded by V_H J558 Family

Idiotypic system	Antigen specificity of idiotype	mAbs express shared idiotopes			
		Designation	Percent inhibition*	Specificity	Frequency
J558-CD3-2	α1-3 Dextran	8I B1	50.3	TG	3/20
		S2-9-2	44.6	MBP	
		UN59-9	46.4	MBP	
IDA23-AIDA23-2	IDA23				0/20
		8I D2	34.7	TG	
PY211-63.4	HA of PR8	6B6	54.4	Sm	3/20
		Y19-10	50.1	RF	
PY206-SN3-9A	HA of X31				0/20
G5-HP20	GAT	H130	41.7	DNA	2/20
		15-32	50.8	MBP	
		10 VA2	44.8	TG	
36-65-AD8	Ars	H130	49	DNA	3/20
		H241	33.1	DNA	

* With 500 ng chromatographically purified antibody.

anti-Sm antibodies were shared by a large fraction of our autoantibody panel. This result is expected, since Lymberti et al. (37) showed previously that there is a high idiotype connectivity among autoantibodies with various specificities obtained from newborn mice. However, our results clearly demonstrated that V_H J558–encoded autoantibodies share idiotypes with antibodies specific for foreign antigens that are also encoded by genes from the same family.

Taking into consideration our small panel of V_H J558$^+$ autoantibodies and the small number of idiotypic systems used in this study, the frequency of idiotype crossreactivity is very high.

However, our data suggest that several pathways can be used for the activation of self-reactive clones. The activation of such clones requires complex immunologic mechanisms, including the activation of Th cells, the expression of Ia antigens on cell surfaces, and the possible exposure of intracellular antigen such as DNA or Sm. Data indicating that antibodies specific for foreign antigen can bind to self antigen (12, 13), combined with our data showing that "bona fide" autoantibodies can bind to foreign antigen suggest that autoreactive clones can be directly activated, in the case of T-independent antigens, or through Th cell cooperation, with T-dependent antigens.

This concept is in agreement with recent data showing that an intestinal infectious agent is responsible for the expansion of clones producing RF in 129/Sv mice (38). The RF synthesis was completely prevented in cesarian-derived and isolator-reared offsprings of RF$^+$ dams.

Anti-Id antibodies are normally produced during an immune response elicited by conventional antigen (30, 40). Therefore, shared idiotopes between autoreactive antibodies and antibodies specific for foreign antigens can be targets for expansion of these autoreactive clones by anti-Id antibodies. Such a mechanism was envisioned by Plotz, who proposed that anti-Id antibodies against antiviral

antibodies can exert destructive effects by crossreactivity with cell-surface receptors (41).

In addition, the presence of IdX on autoantibodies with various specificities can explain the appearance of multiple autoantibodies during systemic autoimmune diseases such as systemic lupus erythematosus or rheumatoid arthritis, or during polyendocrine syndromes.

Studies in progress are aimed at investigating the activation of autoreactive clones by foreign antigens and anti-Id antibodies carrying the internal image of these foreign antigens in order to assess the pathologic significance of the polyspecificity among autoantibodies reported in this study.

Summary

We examined the binding to foreign antigens and the expression of crossreactive idiotypes by a panel of 20 murine monoclonal autoantibodies encoded by V genes from the V_H J558 family. 9 of 20 antibodies bound to foreign antigens such as bacterial polysaccharides, poly(Glu^{50},Tyr^{50}), poly(Glu^{54},Lys^{37},Phe^{9}), arsonate, and lysozyme, known to interact with antibodies encoded by genes from the V_H J558 family. A high proportion of our panel of autoantibodies expressed crossreactive idiotypes originally borne by monoclonal rheumatoid factors, anti-Sm, and anti-DNA antibodies, all encoded by V genes from the V_H J558 family. Some of these V_H $J558^+$ autoantibodies shared crossreactive idiotypes with V_H $J558^+$ antibodies directed against foreign antigens such as influenza virus hemagglutinin, poly(Glu^{60},Ala^{30},Tyr^{10}), arsonate, and dextran.

The implications of these findings are discussed with respect to the process of activation of self-reactive clones.

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