CHEMICAL SYNTHESIS OF IDIOTOPES

Evidence that Antisera to the Same JH$_1$ Peptide Detect Multiple Binding Site–associated Idiotopes

By MICHAEL V. SEIDEN,* BRIAN CLEVINGER,‡ SEAMUS McMILLAN,§ ANTOINE SROUJI,§ RICHARD LERNER,§ AND JOSEPH M. DAVIE*

From the *Department of Microbiology and Immunology, School of Medicine and ‡Division of Biomedical Science, School of Dental Medicine, Washington University, St. Louis, Missouri 63110 and §The Department of Molecular Biology, Research Institute of the Scripps Clinic, La Jolla, California 92037

Structural heterogeneity of variable region domains of immunoglobulins is enormous because of the unique genetic mechanisms for generating antibody diversity. Variable regions of heavy chains (V$_H$)[1] are encoded by three gene segments, V$_H$, D, and J$_H$, that exist in multiple copies in the genome and that recombine randomly upon immunologic commitment (1–3). Similarly, variable regions of light chains are encoded by two gene segments, V$_K$ and J$_K$ or V$_\lambda$ and J$_\lambda$ (4–6). The combinatorial joining of gene segments expands the number of protein products geometrically. Second, the joining process itself is imprecise so that diversity at the junction of gene segments occurs (7, 8). Both combinatorial joining and junctional diversity mechanisms lead to extreme variability in the third hypervariable regions of the heavy and light chains, which are particularly important regions of the binding site. Third, special time-dependent mutational mechanisms lead to single base changes scattered throughout the recombined variable region genes leading to further protein diversification (9–12).

More than 20 years ago, Kunkel (13) showed that the structural diversity expressed by variable region domains could be detected by antisera. These variable region antigenic determinants, called idiotopes, provide simple and highly specific ways of identifying and comparing variable regions and have been widely used in immunological research. Potentially, any part of the variable domains may express idiotopes and, indeed, a wide variety of these determinants have been described, including those located within or outside the binding site, on V$_L$ or V$_H$ domains, and on the complex of V$_L$-V$_H$ (14–17). The totality of

[1] Abbreviations used in this paper: BSA, bovine serum albumin; CFA, complete Freund's adjuvant; C$_H$, heavy chain constant region; CPM, counts per minute; GAC, group A carbohydrate; HA, influenza hemagglutinin peptide; HF, hydrofluoric acid; HV1-M104, first hypervariable region of MOPC 104; IEF, isoelectric focusing; IFA, incomplete Freund's adjuvant; J$_H$, heavy chain joining segment; KLH, keyhole limpet hemocyanin; M104, MOPC 104; 2ME, 2-mercaptoethanol; PBS, phosphate-buffered saline; PC, phosphocholine; RIA, radioimmune assay; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SPA, staphylococcal protein A; T15, TEPC 15; TFMS, trifluoromethanesulfonic; V$_H$, heavy chain variable region.

idiotopes on an individual immunoglobulin, called an idiotype, constitutes a unique set of markers highly characteristic of each protein. Indeed, Kunkel (18) used idiotypic analysis to estimate that the variable region repertoire of humans exceeded 10^7.

Molecules that share idiotypes could be expected to be structurally and even functionally similar and there are many examples where this is the case (9, 17). Antibodies with the same antigen specificity often share idiotypic determinants and this has been shown to reflect common genetic origins and similar structure. In addition, however, molecules that do not share genetic origins, but which have affinity for the same ligand, such as antibodies and T cell receptors specific for the same antigen (19), and antibodies and cell receptors specific for the same hormones (20), have also been shown to share idiotopes. Thus, some idiotopes are functional markers.

In addition to providing useful markers for investigating variable region structure and function, it has become clear that idiotypes may provide an important means of immune regulation. Jerne (21) first called attention to the possibility that idiotopes on lymphocyte receptors may be recognized by other lymphocyte receptors with antiidiotope specificity, forming the idiotype network; antibodies, suppressor T cells, and helper T cells have all been shown to recognize idiotopes and to augment or suppress idiotype production (22–24). Further, antibodies to idiotypic determinants when passively administered result in the elimination of lymphocytes bearing the idiotype (25). If these cells are malignant, antiidiotypic antisera can be an effective therapy (26). Potentially, if the idiotype is shared by a family of autoimmunity-causing clones, antiidiotypic therapy may be curative.

Thus, idiotypic determinants are extremely important from several viewpoints. It is disappointing to realize that the molecular definition of an idiotope has been accomplished in only a few instances (17). Inability to make molecular assignments of idiotype is due, in part, to the complexity of the process of assignment. Required are both monospecific antiidiotypic antibody and families of idiotype-positive and -negative immunoglobulins that are both completely structurally characterized and sufficiently similar/dissimilar so that the correlation of idiotype with structure can be made by inspection. In the few cases in which idiotype expression can be correlated with certain amino acid residues, it is not clear whether the idiotope is present on these critical residues or, alternatively, on others influenced by the critical residues.

To date, idiotypes have been defined following immunization of animals with intact immunoglobulin. Recently, however, synthetic peptides have been used to generate antibodies that bind both peptide and proteins bearing that sequence (27–29). We have applied this strategy to immunoglobulins in an effort to generate site-specific antiidiotypic reagents (30, 31). Here we show that a peptide corresponding to JH_1 of murine V_H regions stimulates a variety of antibodies to immunoglobulin determinants at least some of which are found preferentially on families of antibodies with the same specificity. This indicates that a short, relatively constant part of variable domains actually forms part or all of a multiplicity of determinants.

Materials and Methods

Animals. New Zealand white rabbits were obtained from Isaacs Lab Stock, Litchfield, IL. Sprague Dawley outbred rats were obtained from Harlan Sprague Dawley, Indianapolis, IN.

Peptide Synthesis. Peptides were synthesized manually from free amino acids using standard Merrifield techniques (32). Finished peptides were cleaved from the resin with hydrofluoric acid (HF), characterized by thin layer chromatography, and purified by partition chromatography on G-25 Sephadex. Confirmation of the JH_1 peptide structure was obtained by amino acid sequence analysis. Bombesin, a tetradecapeptide hormone, was obtained commercially (Sigma Chemical Co., St. Louis, MO).

Immunogen. JH_1 and bombesin peptides were coupled via glutaraldehyde (Sigma) to Keyhole limpet hemocyanin (KLH; Calbiochem-Behring Corp., San Diego, CA) as described previously (33). Influenza hemagglutinin peptide (HA) was coupled to KLH through cysteine using *n*-maleimidobenzoyl-*N*-hydroxy-succinimide ester as described elsewhere (34).

Immunization. Rabbits were immunized subcutaneously with 0.5 mg of JH_1-KLH in complete Freund's adjuvant (CFA; Difco Laboratories, Detroit, MI). Rats were immunized with 0.1 mg of JH_{1-KLH} or Bombesin-KLH i.p. Animals were boosted with 0.1 mg of JH_1-KLH in incomplete Freund's adjuvant (IFA; Difco). Control rabbit sera were prepared using a similar protocol and are described elsewhere (35).

Antibody Assays. Indirect and direct radioimmune assay (RIA) have been described previously (36). Briefly, 1 $\mu g/ml$ of antigen in phosphate-buffered saline (PBS) was used to coat 96-well microtiter plates (Cooke Laboratory and Products, Alexandria, VA). After blocking unoccupied protein sites with 1% bovine serum albumin (BSA)/PBS, the antigen-coated plates were exposed to fourfold serial dilutions of anti-JH_1-KLH antisera for several hours. The presence of rabbit antibody bound was detected with 2×10^5 cpm of ^{125}I-goat anti-rabbit IgG (37) (Gateway Labs, St. Louis, MO). Plates were washed and counts bound were determined in a gamma counter.

Indirect RIA was used to measure the specificity of binding. Fourfold serial dilutions of purified inhibitors were mixed with 4×10^5 cpm of ^{125}I-myeloma proteins and exposed to microtiter plates coated with a 1:1,000 dilution of rabbit anti-JH_1-KLH. After 4 h of incubation, the wells were washed and radioactivity was measured.

Protein Purification. Most proteins used in this study were affinity purified on Sepharose 4B columns conjugated with the appropriate ligand as described elsewhere (38, 39). Hybridoma-derived proteins 1-B-10, 6A6, and group A carbohydrate (GAC) 123 were used as concentrated serum-free tissue culture supernatants. J606 was purified from ammonium sulfate precipitates of ascites followed by three consecutive euglobulin precipitations.

SDS-PAGE and Western Blot Transfer. M104 and low molecular weight standards (Bio-Rad Laboratories, Richmond, CA) were denatured and reduced by brief boiling in 1% sodium dodecyl sulfate (SDS) and 1% 2-mercaptoethanol (2ME). Standards and immunoglobulin heavy and light chains were separated by discontinuous SDS-polyacrylamide gel electrophoresis (PAGE) on a 12.5% gel. Separated proteins were electrically transferred to nitrocellulose (Schleicher & Schuell, Inc., Keene, NH) in a transfer box as described elsewhere (40). Efficiency of separation and transfer was determined by amido-blue black stain of the proteins on the nitrocellulose paper.

After transfer, the nitrocellulose was exposed to 1% BSA, 0.05% Tween 20 (Sigma) in PBS. Individual strips were incubated in BSA/Tween/PBS with a 1:2,000 dilution of antisera. After a 1-h incubation with the first antibody, the strips were washed and incubated for 1 h with 2×10^6 cpm of ^{125}I-labeled protein A (Sigma). The strips were washed overnight with BSA/Tween/PBS and exposed to x-ray for 24 h at $-70°C$.

Isoelectric Focusing. Serum antibodies were characterized by IEF in a 0.9% agarose gel (41) (FMC Corp., Rockland, ME) containing 2.5% LKB ampholytes 5–8 and 2.5% LKB ampholyte 8–9.5 (LKB, Gaithersburg, MD). 1–4 μl of sera were applied to prepunched wells between a cathodal wick saturated with 100 mM NaOH or imidazole and an anodal wick saturated with 100 mM phosphoric acid or glycylglycine. Proteins were separated

with 12 W constant power until the voltage was over 1,000 V. Immunoglobulins were precipitated with 18% sodium sulfate for 20 min, fixed with 0.01% glutaraldehyde in sodium sulfate for 20 min, and washed extensively. Fixed gels were incubated for 1–2 h with 10×10^6 cpm of ^{125}I-labeled myeloma protein or normal mouse IgG (42). Gels were then washed for 36 h on a rotary shaker, dried, and exposed to film at $-70°C$ for 1–7 d.

Results

Anti-peptide Sera Bind Both Peptide and Immunoglobulin. Table I shows the sequences and relative positions of the mouse J_H segments in the V_H domain. The N-terminal region of J_H is part of HV3 and intrudes into the binding site. The remainder of J_H lies in the groove separating the V_L and V_H domains (43). The C-terminus provides the link with the CH_1 domain.

A peptide corresponding to JH_1 was synthesized (Table I) and coupled to KLH; rats and rabbits were immunized with the conjugate and their sera tested for antibody. Results achieved with a representative serum are shown in Fig. 1. It is clear that JH_1-KLH induces antibodies that bind both peptide and immunoglobulins (Fig. 1). Anti-JH_1 binds JH_1 peptide best, but also cross-reacts with the JH_2 peptide. Only minimal binding is seen with the 15–amino acid peptide corresponding to the first hypervariable region of MOPC 104 heavy chain (HV1-M104; Fig. 1a). Further, the same antiserum binds substantial amounts of four monoclonal antibodies specific for three separate carbohydrate antigens (Fig. 1b) known to have different variable region structures (9, 17, 44). Western blot analysis shows that the anti-immunoglobulin reactivity is heavy chain specific and is capable of detecting denatured heavy chains (Fig. 2, lane 1). The doublet at 70,000 is the glycosylated and deglycosylated M104 heavy chain as determined by trifluoromethanesulfonic (TFMS) acid deglycosylation experiments (data not shown). The fainter band at ~31,000 is probably a heavy chain breakdown product and has been reported previously (31). The serum shows no activity for light chain (lane 1) or numerous other proteins described in the figure legend (lane 2). A rabbit immunized with KLH-HA (sera 3630) in CFA showed no activity for immunoglobulin heavy or light chain or numerous other proteins (lanes 3 and 4).

Identification of Antiidiotope Antibodies. Direct binding RIA demonstrate that anti-JH_1 sera bind several immunoglobulins, but cannot determine whether one

TABLE I

Amino Acid Sequences of the J_H Segments and Synthetic Peptides

	HV 3														
N	*(97–100F)	101	102	103	104	105	106	107	108	109	110	111	112	113	C
JH₁	TYR-TRP-T YR-PHE-ASP-	VAL-	TRP-	GLY-	ALA-	GLY-	THR-	THR-	VAL-	THR-	VAL-	SER-	SER		
JH₂	ASP------------	TYR-	---------	GLN-	----------	----	LEU-	----	----	---------					
JH₃	ALA-T RP-----ALA-	TYR-	---------	GLN-	---------	LEU-	----	----	---------	----ALA					
JH₄	ASP-TYR- TYR-ALA-MET-----	TYR-	---------	GLN-	---------	SER-	----	---	---------						
J₁₅ₛyₙ	-----------------------													-LYS	
J₂₅ₛyₙ	TYR------------	TYR-	---------	GLN-	----------	----	LEU-	-------------	-LYS						

JH_{1-4} are the four germline J_H sequences used in BALB/c mice (53). Peptides J_{15syn} and J_{25syn} were manually synthesized and characterized by TLC and amino acid composition, and in the case of J_{15syn} peptide, by amino acid sequence analysis.

* Kabat numbers denoting position of J segments in the V_H region. N-terminal position assignments will vary depending on length of D segment.
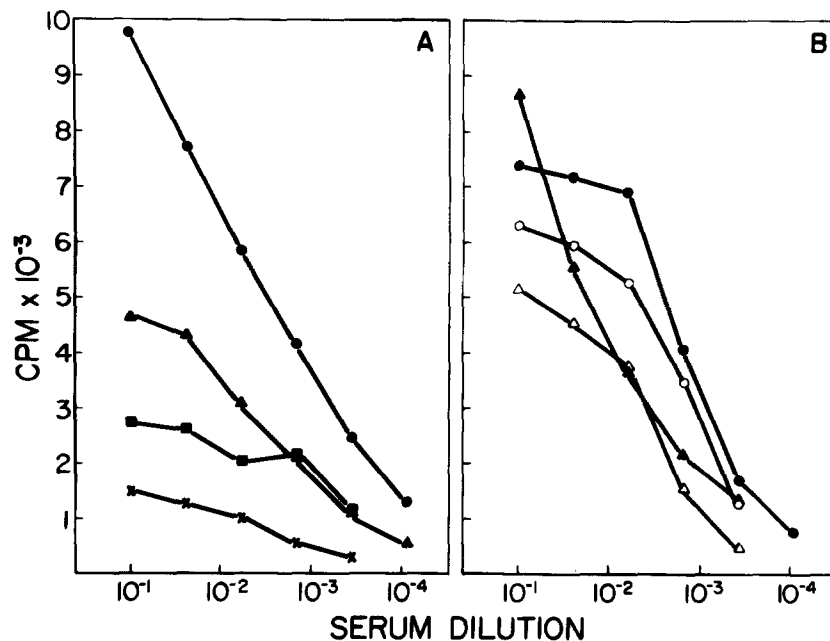
FIGURE 1.   Binding of anti-JH₁ to peptide and various hybridoma and myeloma proteins. A) Binding profiles of JH₁ immunized rabbit sera to various peptides. JH₁ (●); JH₂ (▲); HV1-M104 (■) or BSA alone (×). (B) Binding of anti-JH₁ to T15 (△); M104 (○), GAC34 (▲), or J558 (●). The sequence of HV1 M104 is Cys-Ser-Gly-Tyr-Thr-Phe-Thr-Asp-Tyr-Tyr-Met-Lys-Trp-Val-Lys.

or more specificities are expressed within a given serum. Several approaches were used to examine this question. First, anti-peptide sera from several animals were examined, following isoelectric focusing (IEF) in agarose gels, for binding to various labeled immunoglobulin ligands. This technique permits rapid screening of sera for anti-immunoglobulin reactivity, evaluates heterogeneity of antibody activity, permits comparison between different anti-peptide sera, and, most importantly, allows comparison of the heterogeneity and isoelectric point of antibodies in a single serum that binds different ligands. In Fig. 3, a variety of anti-JH₁ sera (T and numbered samples) and control sera from animals immune to a variety of irrelevant KLH-coupled peptides (a–d and w–z) were focused on four separate gels, which were subsequently exposed to ^{125}I-IgG from normal mouse serum, ^{125}I-J558, an $\alpha\lambda$ anti-dextran antibody, ^{125}I-T15, an αK anti-PC antibody, and ^{125}I-GAC34, a γ_3K anti-GAC antibody. Autoradiographs of these gels show that all anti-JH₁ sera bind normal mouse IgG (panel A). None of 12 rabbit (data for 4 shown) and 4 rat antisera raised to irrelevant peptides have this specificity. The anti-IgG activity is heterogeneous with a slight suggestion of shared bands between sera. Very different patterns are seen when homogeneous immunoglobulin ligands are used. Many, but not all, sera have reactivity to ^{125}I-J558 (panel B); fewer sera are reactive with ^{125}I-GAC34 (panel C) or ^{125}I-T15 (panel D). In most cases, the heterogeneity of the anti-peptide antibodies reacting with monoclonal immunoglobulins is much more restricted than those to normal IgG. Further, antibodies to any one of the monoclonal ligands seem unrelated
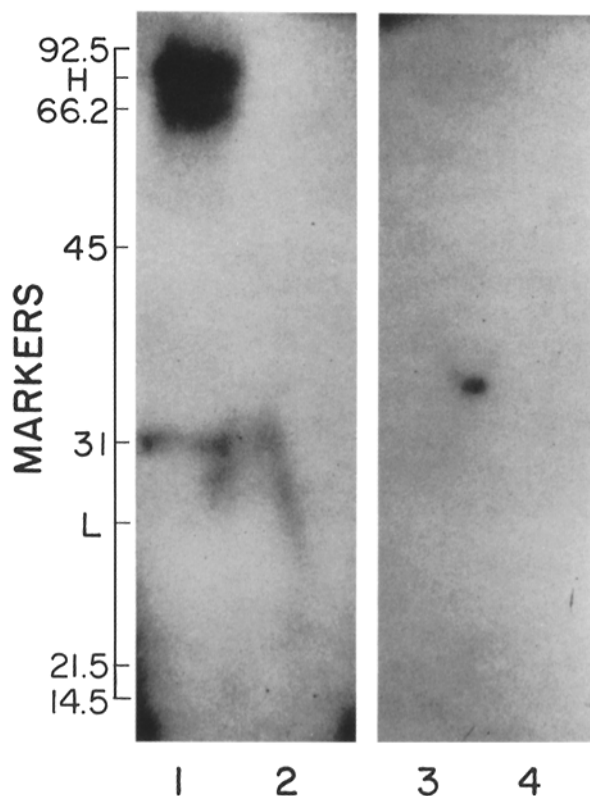
FIGURE 2. Western blot analysis of anti-JH$_1$ sera. Lanes *1* and *2*. 500 ng of MOPC 104 and mol wt standards, respectively, overlaid with a 1:2,000 dilution of rabbit anti-JH$_1$-KLH followed by ^{125}I-SPA. Lanes *3* and *4* same as lanes *1* and *2*, except a 1:2,000 dilution of rabbit anti-HA-KLH control sera was used as first antibody. Molecular weight markers and location of heavy and light chain bands are determined by amido blue-black staining of nitrocellulose strips and are indicated on the left-hand border of the gel. Mol wt standards include phosphorylase B, 92.5; BSA, 66.2; ovalbumin, 45; carbonic anhydrase, 31; soybean trypsin inhibitor, 21.5; lysozyme, 14.5. HA peptide sequence is Cys-Lys-Arg-Gly-Pro-Asp-Ser-Gly-Tyr.

to those reactive with the other monoclonal antibodies. This is best seen in rat serum 11, which binds ^{125}I-J558 very well, yet shows little or no activity for ^{125}I-GAC34. On the other hand, rat serum 4 binds ^{125}I-GAC34 better than ^{125}I-J558. These results indicate that JH$_1$ induces several separate populations of antibodies that bind different determinants in the JH segment and, secondly, that these determinants have restricted distribution.

The second approach to the characterization of anti-peptide antibodies measures the fine specificity of binding to various ligands by competition RIA analysis. In this procedure, the relative capacities of various unlabeled proteins to inhibit the binding of ^{125}I-labeled monoclonal antibodies to anti-JH$_1$ antibodies measures how closely the unlabeled proteins resemble each other with regard to the antigenic determinant(s) expressed on the labeled proband. Table II summarizes the results of these analyses using a single rabbit anti-JH$_1$ serum (serum T) to bind to three ligands, ^{125}I-M104, ^{125}I-T15 and ^{125}I-GAC34. Each protein in
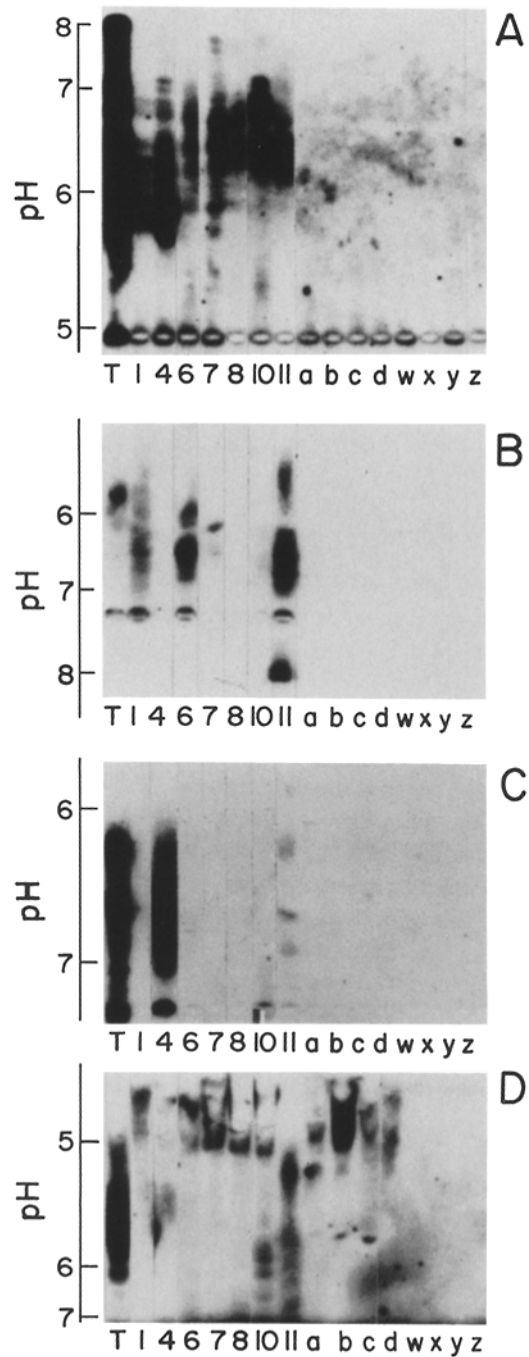
FIGURE 3. IEF patterns of rat and rabbit anti-peptide antisera. Rat and rabbit sera were focused as described in Materials and Methods. Gels were overlaid with (A) ^{125}I-mouse IgG, (B) ^{125}I-J558, (C) ^{125}I-GAC34 or (D) ^{125}I-T15. Lane T contains rabbit anti-JH[1]-KLH used in Fig. 2 and Table II. Lanes 1–11 are sera from individual rats immunized with JH[1]-KLH. Lanes a–d are sera from four rats immunized with bombesin-KLH. Lanes W–Z are sera from four rabbits immunized with HA-KLH. All rat sera demonstrate a nonspecific reactivity with ^{125}I-T15 at low pH.

TABLE II

Expression of JH-Dex, JH-PC, and JH-GAC in Myeloma and Hybridoma Proteins

Speci-ficity	Protein	Isotype	J_H	D	Variable region determinants		
					J_H-Dex	J_H-PC	J_H-GAC
					(relative expression)		
Dextran	M104	IgM, λ_1	J_1	2	100	7	20
	J558	IgA, λ_1	J_1	2	75	4	5
	Hdex1,2,6,7,9, 13,14	IgM, λ_1	J_1	2	100–300	1	ND
	Hdex10	IgM, λ_1	J_1	2	25	9	ND
	Hdex36	IgG$_3$, λ_1	J_1	2	60	<1	<1
	Hdex4,11,24	IgM, λ_1	J_2	2	100	10	2
	Hdex12	IgM, λ_1	J_3	2	200	1	<1
	Hdex31	IgM, λ_1	J_4	2	200	ND	1
PC	T15	IgA,K	J_1	6	<1	100	9
	PG-1	IgM,K	J_1	6	<1	25	5
	M511	IgA,K	J_1	7	4	100	6
	M167	IgA,K	J_1	8	10	140	2
	1-B-10	IgG,K	J_1	?	<1	2	ND
	6A6	IgG$_{2b}$,K	J_1	?	<1	15	ND
GAC	GAC34	IgG$_3$,K	?	?	<1	ND	100
	GAC54	IgG$_3$,K	?	?	<1	<1	120
	GAC57	IgG$_3$,K	?	?	<1	<1	120
	GAC35	IgG$_3$,K	?	?	<1	3	200
	GAC39	IgG$_3$,K	?	?	<1	<1	75
	GAC123	IgM,K	?	?	<1	<1	200
DNP	M315	IgA,λ_2	J_2	3	10	5	6
	29-13	IgG2b,λ_1	?	?	10	ND	ND
	M460	IgA,K	?	?	<1	ND	ND
Levan	J606	IgG$_3$,K	J_3	1	10	ND	100
	M47A	IgA,K	J_3	1	2	100	2

Numerous proteins are examined for their expression of J_H-Dex, J_H-PC, and J_H-GAC. Expression of J_H-Dex, J_H-PC, and J_H-GAC are reported as a ratio of μg of inhibitor required to generate 50% inhibition as compared to standard inhibitors. Values for 50% inhibition by standards are 40 ng GAC34 for J_H-GAC, 270 ng T15 for J_H-PC, and 36 ng M104 for J_H-DEX. D represents the number of residues assigned to this segment.

Table II was tested for the ability to block the binding of each of the three ligands to anti-JH$_1$. The results are clear. All dextran-specific antibodies are capable of blocking ^{125}I-M104 from binding to anti-JH$_1$; no other protein tested had this ability. Similarly, most of the anti-PC antibodies shared a determinant(s) with T15 that was absent from all but one other protein, and all six anti-GAC antibodies expressed a determinant(s) absent from nearly all other proteins. Thus, these three distinct determinants, called J$_H$-Dex, J$_H$-PC, and J$_H$-GAC, are highly associated with families of proteins sharing antigen specificity. Surprisingly, at least two of the determinants were not restricted to the JH$_1$-bearing antibodies.

Discussion

Our goal at the outset of these studies was to produce antibodies specific for the individual J$_H$ segments. Instead, we found multiple determinants involving J$_H$ segments, each highly restricted in its distribution to groups of antibodies

with similar antigen-binding specificity. Previously, Andrews and Capra (45), as well as Schiff and co-workers (46), suggested that idiotopes may be located in the J segment, but could not rule out other portions of the variable domains in their assignments. Our surprise comes not from the realization that J_H segments can participate in idiotypic determinants, but from the multiplicity of separate determinants found within a stretch of 16 amino acids. We believe these results require that conformational changes of J_H residues be induced by other segments of the V_H domain. To evaluate this hypothesis, it is instructive to consider the available structural information pertaining to V_H domains.

Crystallographic studies of the V_H domain have demonstrated that this region is composed entirely of β pleated sheets (43, 47, 48). In the intact Fab, this polypeptide chain folds into a nine-stranded beta barrel in which each of the nine strands is stabilized through hydrogen bonds to neighboring strands. The J_H segment forms the ninth strand in the beta barrel, is in the immediate vicinity of the antigen binding pocket, and interacts through hydrogen bonding with the first (residues 1–14) and eighth (residues 91–100) strands of the barrel. Conformation of the J segment will be dependent on its own primary sequence, influences from neighboring strands, as well as complex interactions with other regions of the V_H, and the C_{H1} and V_L domains. It is conceivable that changes in the length of the D segment, which lies in the hairpin turn of strands eight and nine, may play a crucial role in the conformation of the J segment. This idea gains support from the fact that J_H-Dex is expressed by proteins with short, two-residue D segments, while J_H-PC is found on immunoglobulins with 5–7 residue D segments. Unfortunately, crystallographic data examining the effect of insertion or deletion of residues on the secondary and tertiary structure of similar proteins is very limited. High resolution crystallographic data of the V_H region are limited to three proteins: Kol, New, and McPC 603 (43, 47, 48). The New C_{H1} domain has an additional residue relative to McPC 603 C_{H1} (49) that is accompanied by a beta bend with minimal distortion of conformation. On the other hand, the J segments of New and Kol demonstrate differences in conformation; however, interpretation is difficult since only New is sequenced (50). Finally, structural data from the Rei and Rhi Bence-Jones dimers have demonstrated that the molecules show different conformations in sequence-identical regions because of residue changes in neighboring hypervariable regions (51). Therefore, the fragmentary structural data, at the least, indicate that conformational changes can be expected in the J segment that are induced by other parts of the variable region. Indeed, these putative conformational determinants must accommodate significant changes in primary structure of the J segment itself, because, at least in the case of J_H-Dex, all four J segments express the same putative conformational determinant.

Although it is not possible to decide which parts of the variable domains are important in imparting the conformational changes to J_H, it is interesting that the entire V_H segments of each family of idiotope-bearing molecules are very similar. Each family is encoded by one or several very similar V_H segment genes. This is also true for the anti-levan antibody, J606, that expresses the J_H-GAC determinant. It is conceivable that some "V_H" or idiotype markers actually reside in the J_H region. The 21A5 idiotype recently described by Morahan and col-

leagues (52) bears some resemblance to our J_H-PC marker. Since their determinant is hapten inhibitable and C_H locus-restricted, the idiotype may be located, at least in part, in the JH_1 region. We have found that an anti-JH peptide monoclonal antibody reacts exclusively with μ chains (unpublished results); this suggests that C_H regions may influence the conformation of the J_H segments as well.

We believe that anti-peptide antisera will provide an important approach to the localization of variable region markers. With this technique, one can confidently predict in which section of the variable region an idiotope is located. Furthermore, by synthesizing several overlapping peptides it may be possible to map the location of these idiotopes to a key residue or residues. Further, this technique may also be useful in mapping determinants that have already been defined by classical serology. This has recently been demonstrated with antisera generated against peptides corresponding to the third hypervariable region of M104 and J558 (31). Conventional approaches had tentatively localized the private idiotypic determinants of these two proteins to the D segment. Antibodies directed to peptides corresponding to HV3 were shown to distinguish between the proteins in a predictable way, confirming the previous conclusions. More interesting, however, will be the surprises that emerge from use of this new approach.

Summary

In an attempt to better understand the molecular basis of idiotypy, we have generated several site-specific antisera through immunization of animals with synthetic peptides corresponding to the (JH_1) heavy chain joining segment 1 of the mouse heavy chain variable (V_H) region. These anti-peptide sera identify several idiotypic determinants present on intact hybridoma and myeloma immunoglobulins. Expression of at least three of these idiotopes is correlated with the antigen specificity of the family of immunoglobulins bearing the determinant. Use of synthetic peptides may prove a powerful technique in the generation of molecularly defined antiidiotypic reagents.

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