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Perspectives on Antigenicity and Idiotypy

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INTRODUCTION

Over ten years have passed since the concept of using synthetic peptides to probe antigenicity was first developed [1, 2]. Since then, prominent among the applications of synthetic immunogen technology in biology and medicine [3, 4] is the utilization of synthetic peptides derived from the antigen for vaccine development [5]. The network hypothesis of Jerne [6] offers still another elegant concept for vaccine development. The anti-idiotype concept [7] provides an approach whereby an antigen can be substituted by an antibody possessing characteristics of that antigen. This can be demonstrated by using an anti-idiotypic antibody (Ab2) as a surrogate antigen that can stimulate an antigen-specific immune response [7]. This avenue provides an alternative in cases where the production of antigen based upon molecular biological approaches may not be feasible.

The development of idiotope (Id) derived vaccines rests on the principle of molecular mimicry. An understanding of the structural basis of molecular mimicry could improve the production of idiotype vaccines, moving it from

an experimental state to a rational approach [8]. The importance of molecular mimicry by monoclonal anti-idiotypes (anti-Id) is the ability to make T cell-independent antigens T cell-dependent. For example, anti-idiotypes would allow the presentation of carbohydrate antigens as mimicked by the structure and conformation of the protein surrogate [9]. The mimicking abilities of such "internal image" antibodies also sets the stage for the possibility of producing fully synthetic idiotope vaccines using essential sequence information obtained from idiotope hybridoma antigens. The use of such designed idiotope-derived synthetic peptides would thwart problems associated with the administering of mouse hybridomas to humans [10].

Of fundamental importance in designing new peptide antigens is the faithfulness or fidelity of the molecular mimicry. Antibody-antibody interactions are modulated by their large surface areas, so the complete description for an Id may entail contact points which are close in space but remote in sequence. Depending on the idiotope, there may be two components which contribute to the degree of mimicking fidelity: essential mimicking residues, and contact residues whose complementary interactions lend to the overall association constant for a particular complex formation. This latter component may also play an important secondary role in helping to stabilize a particular structural environment required for full antigenic mimicry. To disentangle these possible effects and ultimately achieve the successful development of a functional antibody or peptide vaccine, it is imperative to fully understand the structure of the antibody molecule, the basis of idiotypic expression in three dimensions and the mechanisms by which large surface areas on proteins modulate protein-protein interactions. These points are addressed in this volume. Here, we present an overview of the salient features of these topics.

THE ANTIGENIC NATURE OF IMMUNOGLOBULINS

Idiotopes represent a particular category of antigenic determinants which can activate clones bearing complementary paratopes through a self-recognition process. This behavior implies that idiotopes are auto-antigens: self-antigens recognized by the immune system. One model proposes that the response to such self-proteins is directed against sequence regions that exhibit the highest evolutionary variability [11]. Therefore, according to this model, sequence-variable regions are antigenic and evolutionarily conserved regions induce tolerance. From a structural perspective, variable, and there-

fore antigenic, regions can tolerate local changes in conformation and should correlate with sequence regions which are flexible and surface exposed [12]. Thus, the combination of intrinsic factors such as mobility and accessibility, and extrinsic host factors such as tolerance, immune response genes, idiotype networking and structural gene repertoire appear to describe protein antigenic structure [11, 13].

Historically, complementary relationships in the recognition properties of immunoglobulins have been attributed to the hypervariable nature of immunoglobulin sequences; complementary determining regions (CDRs) [14]. These regions have been typically associated with the antigen binding site [15] and have shown some correlation with the self-association of light and heavy chains [16, 17]. Conversely, residues which are not classically hypervariable can be complementary in the context of idiotope recognition [18].

SURFACE VARIABILITY ANALYSIS

Considering that the classical views of immunoglobulin (Ig) hypervariability and binding site complementarity may not necessarily be equivalent concepts, it may be appropriate to re-evaluate variability from other viewpoints. A theoretical approach, referred to as surface variability analysis, couples both intrinsic and extrinsic factors of antigenicity by considering the evolutionary variability of protein surface regions [19]. This method characterizes autoantigenic loci in protein families based upon examination of the variability in the hydrophilic properties of evolutionarily variant protein sequences. Surface variability is measured as a function of hydrophilicity and evolutionary sequence variation [19]. For each sequence, hydration potentials defining the affinity of each amino acid side chain for solvent water [20, 21] are averaged over six residues and inverted to make hydrophilic values positive. At each sequence position, the resulting hydrophilicity profiles are averaged to form a consensus value and assayed for variability according to the formula of Wu and Kabat [14]: number of different (hydrophilicity) values divided by the frequency of the most common (hydrophilicity) value. The product of the consensus and variability of hydrophilicity values is used to define a surface variabilty index giving maximal values for surface-exposed sequences which varied significantly during evolution, *i.e.* those likely to form antigenic determinants. Advantages of surface variability analysis over consideration of surface-accessibility to antibodies are 1) a canonical ensemble of structures is evaluated, 2) parameters associated with

the intrinsic factors of protein structure are related to the extrinsic biological factors of evolution that play a role in defining antigenicity, and 3) the distinction shown between strong and weak immunodominant regions correlates with serological trends.

Surface variability analysis of variable domain Ig sequences highlights potential autogenic surface regions, referred to as idiotope determining regions (IDRs). Surface variability profiles (Fig. 1) for a family of 25 mouse and human Ig variable region sequences [22] show that, although the majority of IDRs correspond to hypervariable regions, IDRs also occur in framework regions. It is clear from the profiles that the majority of surface variability in this Ig family resides in the heavy chain. In Figure 2, the antigenic topography of the Fv region is illustrated by mapping surface variability values (classified into 4 categories from Figure 1: most variable, more variable, less variable, and least variable) onto the surface of the 3-dimensional structure of MPC603 [23, 24]. IDRs are depicted by the most brightly colored surface regions (Fig. 2B) contributed by residues with high surface variability (shown with labels in Fig. 2A). Clearly, IDRs cover a continuum of binding sites in the variable region. The large repertoire of IDRs should allow many combinatorial possibilities for idiotope expression in three dimensions, including those formed solely by light chain residues, those formed solely by heavy chain residues, and those formed by residues of both chains. Topographic mapping of one idiotypic system has shown a linear idiotope map spanning from the antigen binding site to the vicinity of the constant region (see Greenspan and Monafo, this issue). Surface variable regions including framework residues may be recognized by more cross-reactive anti-idiotypic antibodies, since fewer CDR residues are involved.

Of the framework residues in the light chain, only 49 and 85 (numbered sequentially according to MPC603) exhibit high surface variability (Fig. 2). Both are isolated sequentially and spatially from the CDRs. In the heavy chain, framework residues with high sequence variability include those adjacent in sequence to CDRs (30, 49, and 99–100), those conformationally adjacent to CDRs on the surface (76–79), and those distant from the CDRs (84, 86, 88). Two clusters of heavy chain framework residues (76–79 and 84, 86, 88) form likely IDRs; residues 76–79 form a protruding beta bend made up of most variable residues, while residues 84, 86, and 88 (outwardly facing residues along a beta strand) form a relatively small flat surface patch of more variable residues. These surface topography and surface variability characteristics suggest that the region emcompassing residues 76–79 forms the most likely IDR outside of the antibody-combining site.

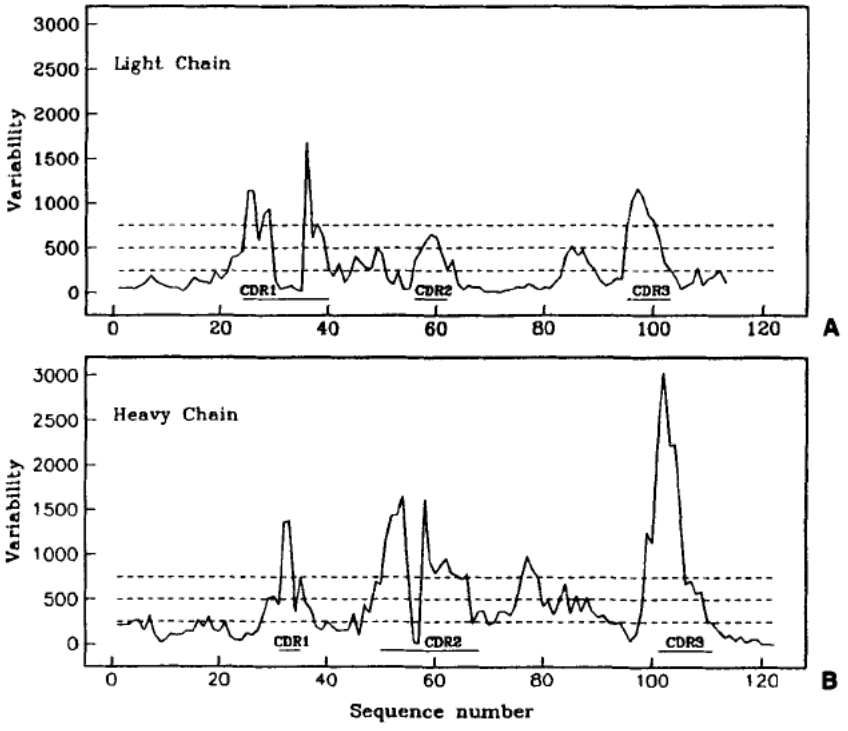
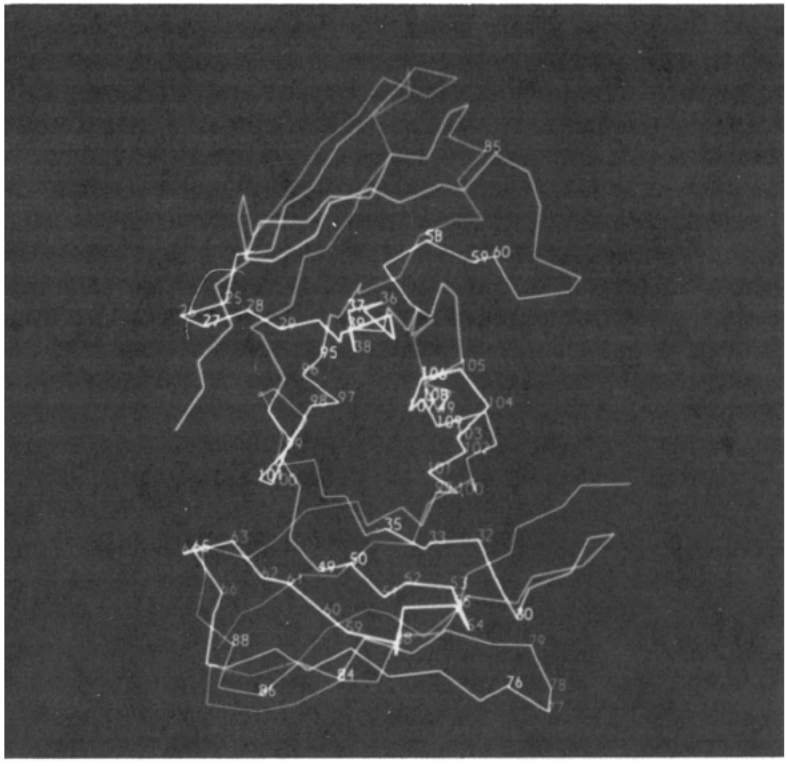
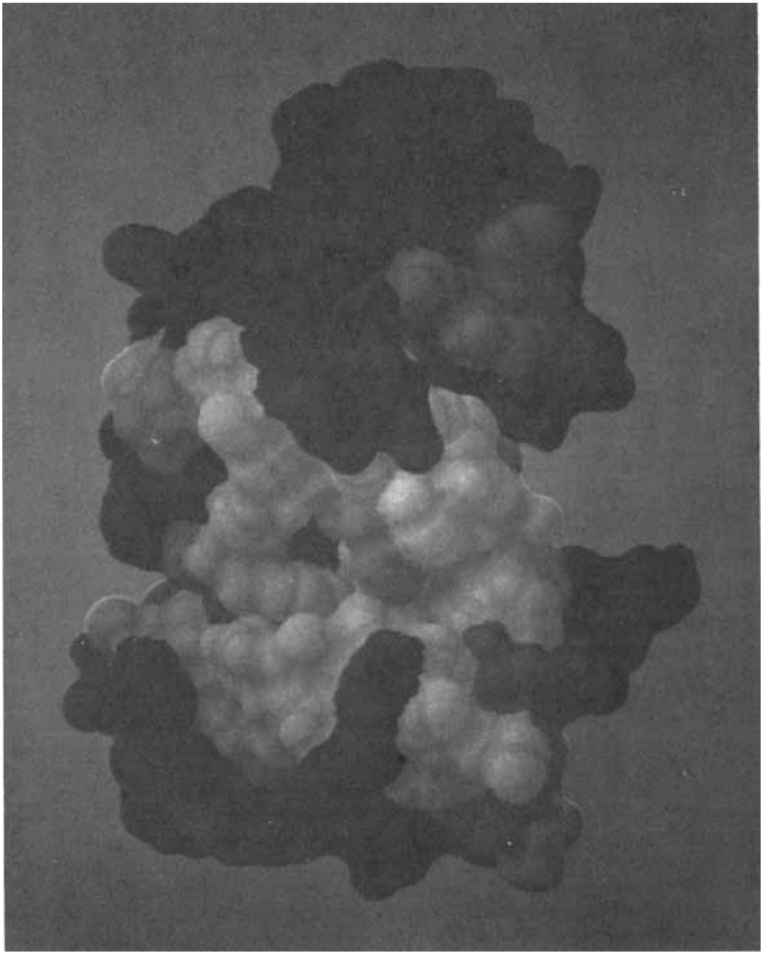


FIGURE 1 Plots of the linear relationship of surface variability to sequence position in the variable regions of the light (A) and heavy (B) chains from 25 mouse and human immunoglobulins [22]. Residues are numbered sequentially to match the sequence of MPC603. Variability is averaged over six residues and plotted at the third position to allow appropriate mapping onto the three-dimensional structure (see Fig. 2A). Complementarity-determining regions are shown by horizontal bars: light chain CDR1 (residues 24–40), CDR2 (56–62), and CDR3 (95–103) and heavy chain CDR1 (31–35), CDR2 (50–68), and CDR3 (101–111). Long dashed horizontal lines separate the four categories of surface variability used to color code Figure 2B. The residues included in each category for the light chain: most variable (residues 25–26, 28, 29, 36, 38, 96–100), more variable (27, 37, 39, 49, 58–60, 85, 95, 101), less variable (22–24, 40, 42, 45–48, 50, 56–57, 61–63, 84, 86–89, 102–103, 108, 112), and least variable (1–21, 30–35, 41, 43–44, 51–55, 64–83, 90–94, 104–107, 109–111, 113–115); and for the heavy chain: most variable (32–33, 51–55, 58–64, 66, 77–79, 99–105), more variable (30, 35, 49–50, 65, 76, 84, 86, 88, 106–109), less variable (4–5, 7, 18, 28–29, 31, 34, 36–37, 45, 47–48, 68–69, 71–75, 80–83, 85, 87, 89–91, 98, 110–111), and least variable (1–3, 6, 8–17, 19–27, 38–44, 46, 56–57, 67, 70, 92–97, 112–122).



A

FIGURE 2 Computer graphics images of immunoglobulin surface variability mapped onto the light and heavy chain variable domains of MPC603. Labeled alpha carbon backbones (A) and solid external molecular surfaces (B) are shown in the same orientation, looking down into the combining site from the solvent. The alpha carbon backbone (A) of the light chain (top) is shown in blue with yellow CDRs and the alpha carbon backbone of the heavy chain (bottom) is shown in purple with green CDRs. In both the light and heavy chains, residues with high average surface variability are labeled (A) by number at the alpha carbon position in yellow (more variable) and red (most variable). The solid external molecular surface (B) is color coded by the surface variability (defined in text and shown in Fig. 1) using a radiating body color scale with increasing variability corresponding to increasingly brighter color. The highly (most and more) variable regions (lightest colors in B) are defined to be potential IDRs; the majority are also CDRs. The most prominent highly variable surface region outside the CDRs is formed by heavy chain residues 76–79 (bottom right), which are conformationally adjacent to CDR2. Other framework residues with high surface variability are light chain 49 (behind) and 85 (upper right), heavy chain 30, 49 and 99–100 (adjacent in sequence to CDR1, CDR2, and CDR3,



B

respectively), and heavy chain 84, 86, and 88 (lower left). The backbone is displayed using the graphics programs GRAMPS [25] and GRANNY [26]. The molecular surface, defined by mathematically rolling a probe sphere (1.4 Å radius) representing a solvent water molecule over the van der Waals surface of the protein, is calculated and displayed using the programs AMS and RAMS [27, 28]. Crystallographic coordinates [23, 24] are taken from the Brookhaven Protein Data Base [29]. (See Color Plates I and II in the color section of this issue.)

IDIOTOPE/PARATOPE OVERLAP

IDRs agree quite well with idiotope locations deduced from sequence analysis (Table I; see also Chen, *et al.*, this issue). IDR areas have been shown to be surface-exposed (see Novotny in this issue). Surface variability analysis has implied that 1) there are more IDR than CDR loci and 2) all CDR loci are also IDR loci. Structural and functional implications of this relationship between CDRs and IDRs have been discussed [22] (see also Stevens and Schiffer, this issue). The overlap between idiotopes and paratopes [22] implies that idiotopes can participate as binding sites; the dual roles of binding to a determinant or being bound by another antibody may be expressed simultaneously by the same topographic site. Consequently, sites on Igs may not be easily characterized functionally [22]. In support of this

TABLE I

Correspondence of Experimentally Deduced Idiotype Locations and Predicted IDRs

System	Experimental Position	Predicted Position	References
Anti-Dextran	H96–97	IDRE-H	30, 31
	H53–54	IDRC-H	32, 33
	HFR3	IDRD-H	18
Anti-Phosphocholine	HV3	IDRE-H	34
Anti-Galactan	H96–97	IDRE-H	35
	H53	IDRC-H	
Ars-A	L30	IDRB-L	36
	H59	IDRD-H	
	HV3	IDRE-H	
	LV3	IDRG-L	
Anti-Inulin	L53	IDRD-L	32
	L56	IDRD-L	
	L53	IDRD-L	
B lymphocyte	H94–99	IDRE-H	37
Anti-4-hydroxy-3-nitro-5-idiophenyl	HV3	IDRE-H	38
IgM-RF	H94–99	IDRE-H	39–44
	L28–31	IDRB-L	
	L53	IDRD-L	
	HV2	IDRC-H	
	LFR2	IDRC-L	45
	LFR3	IDRE-L	

Experimental positions correspond to H (heavy), L (light) chain designation with standard numbering scheme [93]. HV and LV designation for heavy chain variable and light chain variable regions 1 to 3, respectively [93]. The nomenclature for corresponding IDR predicted positions are from [22].

notion is the recent description of two antibody structures, one with a unique V-V packing arrangement (see Stevens and Schiffer in this issue) and the other emphasizing the expanse of contact area across the surface of the antibody [46].

Idiotypic and anti-idiotypic interactions have been classified according to the location of the idiotope on the Ig surface relative to their paratope [6, 47–50]. Definitions derived from such topographic relationships are arbitrary in that an idiotypic antibody (Ab1) that is defined for one system can be an anti-idiotypic antibody (Ab2) in another system. The fact that the same antibody can bind epitopes while presenting itself as an antigen further emphasizes the correlation between paratopes and idiotopes.

STRUCTURAL VERSUS TOPOGRAPHIC DETERMINANTS

The phenomenon of molecular mimicry would appear to suggest a chemical/structural equivalence between the antigen and anti-Id antibody. However, similar three-dimensional surface environments do not imply that the secondary and tertiary folding pattern of the antibody and anti-idiotype are the same. For protein antigens, the identification of homologous sequence regions between the nominal antigen and anti-Id does not ensure the identification of the "internal image," since different three-dimensional environments influence the folding patterns of the related sequences [51, 52]. On the other hand, the framework (FR) region of an antibody represents a simple beta sheet scaffold onto which binding sites may be built, implying that the structure of CDRs is relatively independent of the FR context. The conformation of CDR loops between beta strands depends on loop sizes and specific interactions between the loop and the beta sheet. Studies on the conformational attributes of the antibody molecule [53–57] have emphasized antigen binding. Here, we are interested in how the folding patterns if Igs can be related to the folding pattern of nominal antigens, in particular, whether certain CDRs and FR regions influence the expression of idiotopes in terms of sequence or backbone conformation. Historically, determinants that seem to be affected by single residue changes have been described as topographic, because the immune system must be responding to local changes in the surface topography of the molecule [58]. Alternatively, determinant that involve changes in the backbone conformation were called conformational determinants or structural determinants [59]. Neither of these definitions preclude that the determinants may be discontinuous. An

important difference in the two determinant features is that a synthetic peptide may not be able to adopt a particular conformational determinant. The subtle difference between these determinant types may also influence the expression of cross-reactive idiotopes which certainly play a role as regulatory idiotopes [18].

The nature of antibody-antibody interactions implies that extended areas of the protein surface contribute to binding, leading to a multisite interaction model for idiotope recognition. The required thermodynamic conditions for such interactions can be achieved via several sources [13] and can be quite precise in that the interactions can be affected by a single amino acid change [60–63]. Experimental observations idicate that both sequence and conformation dictate an immune response [64, 65]. In either case, the degree of spatial adjustments in the molecular partners results in different free energies of association for any given antibody with any other given antibody or antigen. The overall energetics of the interaction is determined by the free energy cost (G) of any conformational changes experienced by either the antigen (anti-Id) or the antibody upon complex formation. At the molecular level, the relative energy cost of the spatial displacement of interacting groups is intrinsically associated with the relative mobility of a region. The concept of macromolecules as flexible entities and range of the dynamical nature of proteins has been extensively discussed [66–72].

Determinants are often comprised of discontinuous parts of an antigen. In the crystal structure of Amit and coworkers [46], the lysozyme determinant is made up of two stretches of polypeptide chain comprising residues 18 to 27 and 116 to 129. Surface variability analysis of lysozyme [19] is in relatively good agreement, identifying the majority of residues in this epitope (residues 14–21 and residues 115–126) as autogenic loci. While CDR3 of the heavy chain of the antilysozyme forms the principle contacts, all six CDR regions are involved in contacting lysozyme. Molecular modelling studies of lysozyme-antilysozyme complexes [73] also suggest that the loop region epitope (residues 57–84) of lysozyme is described by autogenic loci [19] centered on positions 67–79. In these complexes all six CDR regions are also involved in defining contacts.

Idiotopes (and antigenic determinants in general) have been localized by evaluating the reactivity of antibodies to the protein with synthetic peptides derived from different parts of a protein [33] (see Chen, *et al.*, this issue). Small peptides are thought to exist in a multiplicity of transient conformational states in dynamic equilibrium, in contrast to the relatively stable structure of a protein in solution [74]. Nevertheless, small peptides can

induce antibodies with a sequence and structural requirement for binding antigen comparable to antibodies raised against the native protein [3]. Whether this is because certain peptides adopt a conformation similar to that found in the native protein [75] or this results from induced fit remains to be seen. The energetic considerations which mediate antipeptide-peptide complexes and the relationship to antipeptide-antibody interactions with the parent molecule have been discussed [13, 64, 65].

The degree of specificity of a particular complex, as well as the possible description of epitope regions, relies on the consorted, dynamical nature of an antigen-antibody interaction [12, 76, 77]. However, in some circumstances a restriction in the conformational freedom of immunizing peptides will result in antibodies with the same specificity as those induced by the proteins themselves [78, 79]. This result focuses on the supporting structural role of regions remote in sequence to a primary epitope center. Short synthetic linear peptides may not account for such environmental contributions [80]. The influence of a tertiary environment on the natural conformations of short peptide segments is clearly evident, in that identical sequence segments in native proteins can have different conformations [51, 52]. The possible fine specificity in antigenic recognition by B cells apparently extends to T cells, where different determinants can be formed by the same peptide and Ia molecule [81].

MIMICRY OF CONTACT RESIDUES

The possible ways in which anti-idiotypic antibodies can mimic antigens has been summarized [82]. Sequence homology with the protein antigen has been suggested for the mimicking capabilities of monoclonal antibodies in the reovirus system [83]. Sequence analysis of a hybridoma that mimics the reovirus antigen indicates sequence homology in an expected IDR of the kappa light chain [83]. A linear synthetic decapeptide derived from the anti-Id (LV2;hypervariable region 2 of the light chain;CDR2), which is homologous to reovirus in five positions and has three conservative substitutions is capable of inducing biological responses similar to the reovirus antigen as well as the anti-Id [83 and M. Greene, personal communication]. Essential contact residues which define this reovirus epitope appear to be retained in the Ab2 in a homologous, linear fashion. In a random fashion, the likelihood of homologous matching of five residues in this hypervariable region, as evidence in the reovirus system, is $7:20^5$. Although such homology would be

rare in randomly generated sequences, analysis of viral protein sequences suggests that rudimentary viral epitope sequences are established within the Ig germ lines (Kieber-Emmons and Köhler, unpublished).

In Table II, light chain sequences of the crystallographically known Ig structures are aligned with those of the proposed reovirus epitope and its mimicking anti-Id. The resulting homology points out the possible structural similarities between the mimicking monoclonal 87.92.6 and MPC603 in this region. In Table III, heavy chain-sequence comparisons with reovirus indi cate additional homology including the identical reovirus sequence (NSYSGS). Comparison of the crystallographic structures of HV2 (hypervariable region 2 of the heavy chain;CDR2) regions from NEWM and MPC603 also indicates that the conformational properties are highly constrained in spite of sequence differences.

The alignments in HV2 and LV2 provide an opportunity to compare the conformational properties of the sequences of the proposed reovirus epitope and the mimicking monoclonal. The sequences in the two tables can be considered as structural variations of beta bends. The structural similarities provide a data base [64] to help elucidate residues essential for antibody 87.92.6 recognition and binding and residues integral to the idiotope because they stabilize secondary structure essential to the presentation of the antigenic site.

The identification of sequence similarities between an antibody and an antigen is nongermane in the analysis of anti-idiotypic antibodies mimicking carbohydrates or haptenic antigens. For these cases, conceptual physical/chemical models (*e.g.* the proper alignment of functionally reactive groups, or of residues with van der Waals interaction tendencies, shape or other physical attributes resembling those of the antigen) must be invoked as an initial basis for mimicry of nonprotein antigens. Such models must also be examined in cases where the mimicking antibody and protein do not exhibit sequence homology. Thus, the degree of chemical or sequence similarities will certainly affect the degree of mimicry fidelity or faithfulness.

An anti-T15 hybridoma, 4C11, is capable of inducing biological effects similar to those induced by phosphorylcholine (PC) [85, 86]. Competitive bindings assays have shown that PC is a successful inhibitor of 4C11-T15 binding. Crystallographic analysis of the PC-binding antibody MPC603 and PC-binding studies provide a basis for examining potential contact residues in the variable domains of 4C11 that could provide chemical mimicry of PC. Conformational attributes of model peptides interacting in a manner similar to PC with MPC603 suggests that the tetrapeptide Lys-Gly-Gly-Asp is

TABLE II

Alignment of Structurally Known LV2 Regions with Reovirus and Anti-Id

MPC603	Leu	Leu	Ile	Tyr	Gly	Ala	Ser	Thr	Arg	Gln	Ser	Gly
Reovirus	Ile	Val	Ser	Tyr	Ser	Gly	Ser	Gly	Leu	Asn	Trp	Arg
87.91.6	Leu	Leu	Ile	Tyr	Ser	Gly	Ser	Thr	Leu	Gln	Ser	Gly
NEWM	Leu	Leu	Ile	Tyr	Arg	Asp	Asp	Lys	Arg	Pro	Ser	Gly
KOL	Leu	Leu	Ile	Tyr	Arg	Asp	Ala	Met	Arg	Pro	Ser	Gly

The sequences for MPC603, NEWM, KOL, and reovirus were obtained from Protein Identification Resource [84]; the sequence for 87.91.6 is from [83].

TABLE III

Alignment of Selected HV2 Regions with Reovirus and Mimicking Anti-Id

Reovirus	Gln	Ser	Met	—	Trp	Ile	Gly	Ile	Val	Ser	Tyr	Ser	Gly	Ser	Gly	Leu	Asn
36–60	Asn	Lys	Leu	Glu	His	Met	Gly	Tyr	Ile	Ser	Tyr	Ser	Gly	Ser	Thr	Tyr	Tyr
NEWM	Lys	Gly	Leu	Glu	Trp	Ile	Gly	Tyr	Val	Phe	Tyr	His	Gly	Thr	Ser	Asp	Asp
MPC603	Lys	Arg	Leu	Glu	Trp	Ile	Ala	Ala	Ser	Arg	Asn	Lys	Gly	Asn	Lys	Tyr	Thr
87.91.6	Gln	Gly	Leu	Glu	Trp	Ile	Gly	Arg	Ile	Asp	Pro	Ala	Asn	Gly	Asn	Thr	Tyr

Sequences for reovirus, 36–60, NEWM, and MPC603 are from the Protein Identification Resource [84]; the sequence for 87.91.6 is from [83].

representative of the major antigenic determinant of 4C11 which interacts with T15 [87]. Competitive inhibition assays have shown that the synthesized model tetrapeptide is capable of inhibiting 4C11-T15 binding, though not as effectively as PC [87]. Sequence analysis [88] and molecular modelling of 4C11 indicates that a loose chemical mimicry of the dipolar character of PC localized in CD2 of the heavy chain may have a dominant role in determining the antigenic topography of the hybridoma. For a small haptenic antigen like PC, which is being mimicked by a comparatively large molecule like an antibody, mimicry may be mediated by appropriately placed contact residues that reside on different parts of the hybridoma. The mode of interaction of an antibody with a small hapten and with a large protein are different. So there may be multiple ways in which mimicry can be realized. Residues of anti-PC antibodies that interact with PC may be utilized in 4C11 binding, but other residues of the anti-PC antibodies that do not contact PC may now contact 4C11. Anti-PC antibodies with different sequences may not bind 4C11 or may bind with very low affinity, due to the proximity of noncomplementary residues over a large surface area in the complexes.

SUMMARY

The recent crystal determination of a lysozyme-antilysozyme complex provides a three-dimensional prototype of the manner in which contacts in idiotype-anti-idiotype interactions may be realized [46]. Such interactions can be approximated by two complementary "flat" surfaces. Each IDR (autoantigenic locus) location might provide a particular recognition feature between two interacting partners. The combinatorial manner in which IDR domains are recognized by anti-idiotypic antibodies describe the repertoire of private and public (crossreactive) idiotopes of an antibody.

Several interesting features emerge from consideration of the Ab contact residues in the crystal structure. First, framework residues are implicated in contacting the antigen: Thr 30 (FR1) of the heavy chain and Tyr 49 (FR2) of the kappa light chain. Both of these residues lie within predicted IDRs [22]. Framework regions have recently been suggested to be involved in several anti-idiotypic systems [18, 45], although such regions have, in the past, been disregarded based solely upon sequence analysis. The surface variabilty analysis, which identifies the repertoire of complementary interacting surfaces, depicts the immunoglobulin as having more variability than generally

thought. This variability may also extend to T cell receptors since T cell chains express an extensive surface variable repertoire similar to that of the immunoglobulin light chains (Kieber-Emmons and Köhler, unpublished). Second, the D region plays a critical role in the generation of the anti-lysozyme combining sites. Similarly, the D segment makes up the largest component of an IDR [22]. Third, while the CDR3 of the heavy chain contributes most to the antibody-lysozyme complex it is not the most surface-exposed (see Novotny, this issue). Nevertheless, surface variability analysis indicates that this region is generally immunodominant [22] which is also observed experimentally [33]. Together, these results indicate that perhaps certain IDR regions are intrinsically more antigenic.

Idiotypic structures must be accessible for antibody recognition and binding. From a structural viewpoint, a single antibody molecule has a continuum or several different combining sites [89]. Subsequently, a single residue can be contained in several overlapping idiotypic determinants. Surface variability analysis suggests that the hypervariable regions of Igs provide a diverse idiotope repertoire that can be utilized for binding. Monoclonal antibodies have been shown to have multiple specificities [90–92] and this capacity for multiple binding is also intrinsic to the definitions that have emerged for anti-idiotypic antibodies.

From an application perspective, although vaccination against bacterial and viral diseases has been a major achievement of immunology, there still is the need for the development of safe and effective vaccines. Frequent problems in vaccine development are the lack of a source of antigen and the danger associated with live vaccines that are insufficiently attenuated. Even heat-killed bacteria have caused adverse reactions due to toxic bacterial products. The use of idiotope vaccines can overcome many of these problems. By using monoclonal antibodies (or derived peptides) as the idiotope vaccine, a pure and readily obtained source of antigen is provided. Since no pathogen-derived material is used, the problems seen with live or heat-killed vaccines are absent.

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