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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

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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-

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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

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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.

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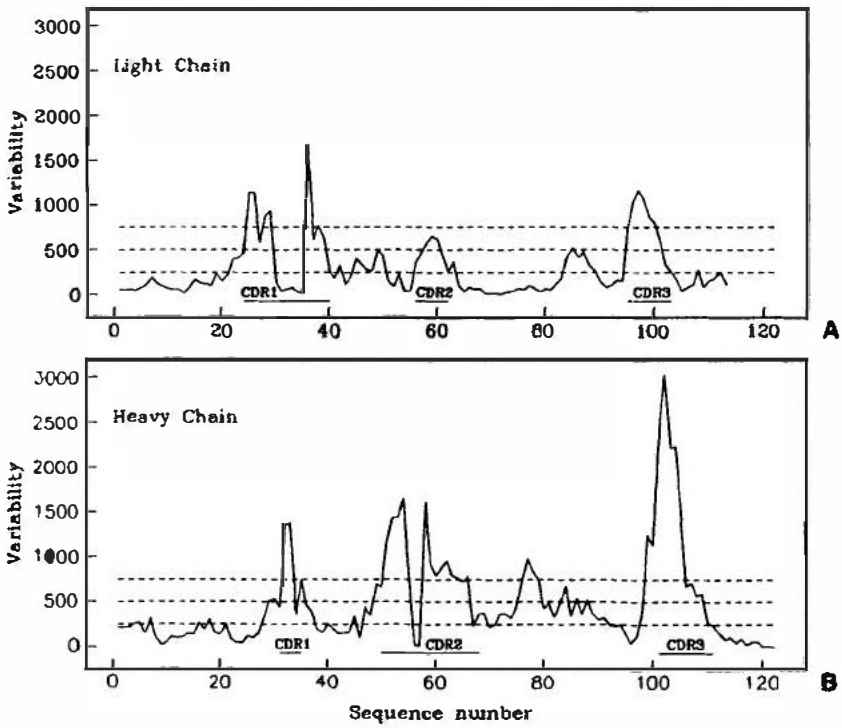

FIGURE 1 Plots of the linear relationship of surface variability to sequence position in the variable regions of the light (A) and heavy (B) chains from 25 mouse and human immunoglobulins [22]. Residues are numbered sequentially to match the sequence of MPC603. Variability is averaged over six residues 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).

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