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Functional Validation of Ligand Mimicry by Anti-Receptor Antibodies: Structural and Therapeutic Implications†

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Several anti-receptor monoclonal antibodies have been described that compete with the ligand for receptor binding. The possibility that structures within the hypervariable or complementarity-determining regions (CDR) of these antibodies might display similarity with the receptor ligand has been documented, thereby validating this immunologic approach to explore ligand–receptor interactions.

The Antibody-Combining Region. A unique feature of immunoglobulins is the enormous diversity of their variable region segments and their ability to interact with an unlimited number of determinants. The antibody-combining region of antibody molecules is obtained within the variable regions of the heavy and light chains. Each heavy or light chain variable region is subdivided into four framework regions separated by three hypervariable regions or complementarity-determining regions (CDRs) (Figure 1). Recombination of gene segments allows for the formation of the variable region of an antibody and accounts, in part, for antibody diversity (Leder, 1982). For instance, any one of a few hundered variable (V) region segments may recombine with one of several diversity (D) region segments (within heavy chains) and one of several joining (J) region segments making a single variable region. In addition to combinatorial diversity, the CDRs accumulate somatically acquired point mutations that may enhance antibody affinity (Wysocki et al., 1986). The CDR regions are reverse turns and, in some cases, conventional β-loops, allowing for free amino acids within the loop or turn to interact with an antigen (Figure 1, inset). The combination of interactions between the CDR regions and the antigen comprises the antibody binding site.

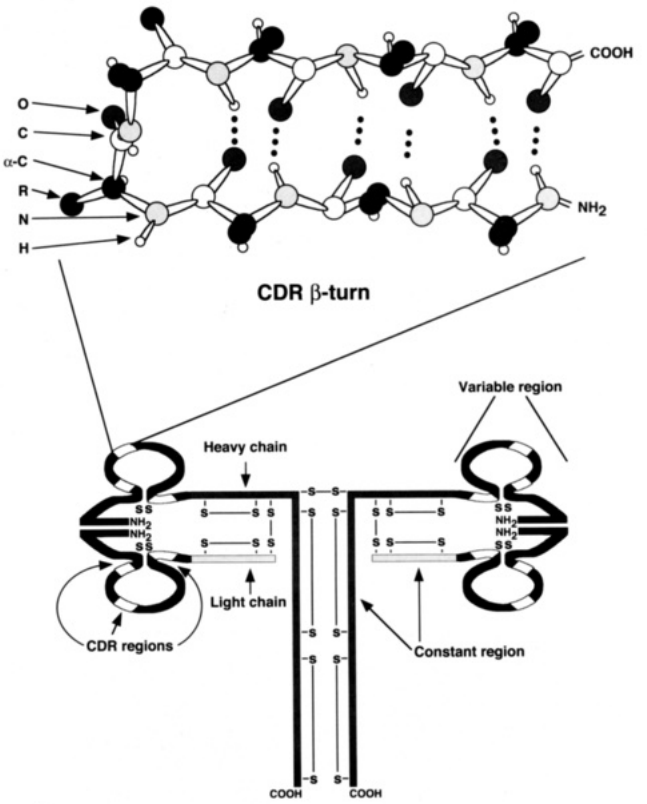

FIGURE 1: Structure of an antibody molecule and CDR region. The structure of an antibody molecule is shown including heavy and light chains, variable and constant region portions, and disulfide bonds. CDR regions are highlighted, and the β-turn structure of a typical heavy chain second CDR region is shown in the inset.

Generation of Anti-Receptor Antibodies. Anti-receptor antibodies have been prepared either by isolating antibodies directed against the receptor or by generating antiidiotypic or autoantiidiotypic antibodies (Cleveland & Erlanger, 1986).

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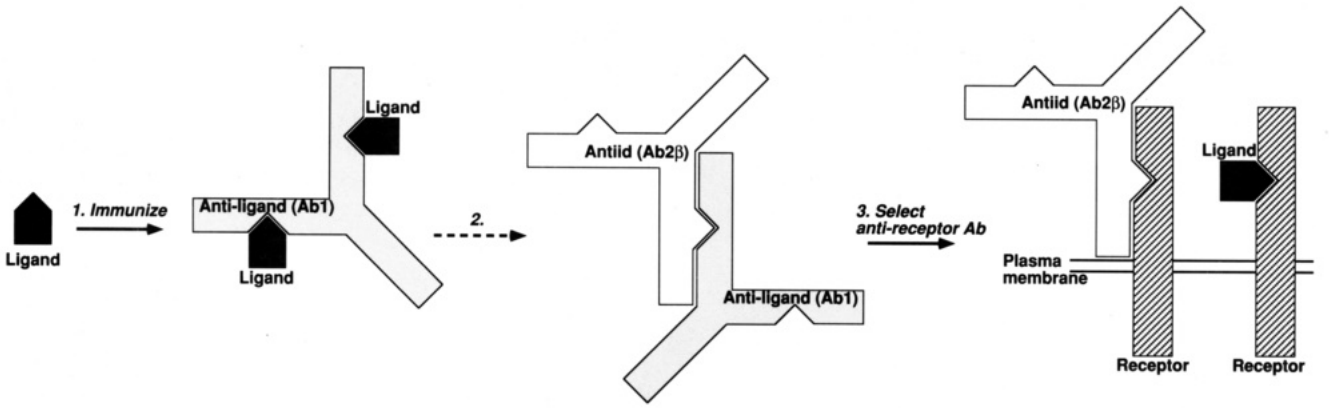

FIGURE 2: Isolation of an antiidiotypic anti-receptor antibody. Step 1: ligand is used to immunize animals and generate anti-ligand antibodies [Anti-ligand (Ab1)]. Step 2: in the autoantiidiotypic approach, antiidiotypic antibodies [Antiid (Ab2β)] directed against the anti-ligand antibodies arise spontaneously in the same animal. Step 3: specific antiidiotypic antibodies that interact with the receptor are selected using biochemical criteria.

Table I: Anti-Receptor Monoclonal Antibodies with Sequence Similarity to the Ligand[a]

receptor	ligand	monoclonal antibody	type	CDR
reovirus (sialic acid)	reovirus	87.92.6 (IgMκ)	antiid	L2 (6/7)
fibrinogen (GP IIb/IIIa)	fibrinogen	PAC1 (IgMκ)	primary	H3 (6/8)
TSH receptor	TSH	4G11 (IgMκ)	autoantiid	L1 (10/13)
TSH receptor	TSH	D2 (IgMκ)	autoantiid	H2 (12/14)

[a] Shown are receptors, receptor ligands, and anti-receptor monoclonal antibodies which demonstrate sequence similarity with the ligand in one of the CDR regions and how they were raised (antiid, antiidiotypic antibody against a primary anti-ligand antibody; primary, primary anti-receptor antibody; autoantiid, antiidiotypic anti-receptor raised directly after immunization with ligand). Under monoclonal antibody, IgMκ indicates the IgM heavy chain and κ light chain. Under CDR, the specific CDR (L, light chain; H, heavy chain; 1, 2, and 3, specific CDR region) and the degree of amino acid similarity with the ligand (in parentheses) are given.

Early on, it was suggested that variable region determinants could imitate determinants on unrelated molecules (Lindemann, 1973; Jerne, 1974). Subsequently, antiidiotypic antibodies in which the combining site of the antiidiotypic antibody mimics the original ligand have been isolated and extensively studied [reviews: Bona et al. (1968); Langman and Cohn (1986); Kohler et al. (1989); Nisonoff (1991)]. A fraction of antiidiotypic antibodies (Ab2β) actually interact with the combining region of the primary antibody (Ab1) directed against an antigen (see Figure 2 in which the ligand is the antigen). Only the combining site of this group of antiidiotypic antibodies would mimic the original ligand at the level of primary or secondary structure and thus form an internal image of the ligand.

Thus, antiidiotypic antibodies prepared by immunization with specific anti-ligand antibodies can be selected for their ability to interact with the specific receptor and block ligand binding by the receptor. Alternatively, using the autoantiidiotypic approach, a ligand can be used to raise an anti-ligand antibody (Figure 2), and within the same animal, an antiidiotypic antibody which reacts with the anti-ligand antibody may arise. Some of these antiidiotypic antibodies react not only with the anti-ligand antibody but also with the receptor combining site, thus mimicking the binding of the ligand.

Properties of Anti-Receptor Antibodies That Interact with the Ligand Binding Site. Initially, naturally occurring antiidiotypic anti-receptor antibodies were detected in the sera of patients with insulin-resistant diabetes, Graves disease, and myasthenia gravis (Flier et al., 1976; Sege & Peterson, 1978; Hall et al., 1975; Dwyer et al., 1983). Subsequently either primary or antiidiotypic antibodies have been isolated that are directed against a wide variety of receptors including members of the integrin family, receptors with tyrosine kinase activity, receptors using sialic acid to mediate binding, adrenergic and thyroid hormone receptors, and other receptors (Shattil et al., 1985; Soos et al., 1989; Wu et al., 1989; Zhang & Roth, 1991; Noseworthy et al., 1983; Cleveland & Erlanger, 1986; Hill & Erlanger, 1988; Wasserman et al., 1982; Courand et al., 1985). The most characteristic property of anti-receptor antibodies that bind to the ligand binding site is their ability to specifically inhibit ligand binding by the receptor and, conversely, that the ligand inhibits binding of antibody to the receptor. It is not necessary that the antibody mimic the ligand at the level of biologic properties, as signaling might require a specific conformational change in the receptor that cannot be achieved by antibody binding. It is also not necessary that the antibody inhibit all biological properties of the ligand. If the ligand has multiple contact points, only one of which is blocked by the antibody, some of the functional properties of the ligand could be maintained in the presence of the anti-receptor antibody.

Well-characterized monoclonal anti-receptor antibodies that interact with the ligand binding site have been identified for the reovirus receptor (Bruck et al., 1986), fibrinogen receptor (Shattil et al., 1985; Taub et al., 1989), and TSH receptor systems (Hill et al., 1987; Taub et al., 1992) (Table I). Monoclonal antibody 87.92.6 was selected using the antiidiotypic approach, by immunizing with 9B.G5, an antibody directed against the reovirus type 3 hemagluttinin. This 87.92.6 was shown to mimic the biological properties of the reovirus hemagluttinin including inhibition of DNA synthesis and T-cell activation and to inhibit reovirus binding to 9B.G5 and to reovirus receptor. Also, 87.92.6 was shown to bind to the reovirus receptor on the cell surface, and by these criteria it was thought to interact with the reovirus receptor at or near the binding site for the type 3 hemagluttinin (Bruck et al., 1986).

PAC1 is a primary IgMκ murine anti-platelet monoclonal antibody that was selected for its ability to interact with the

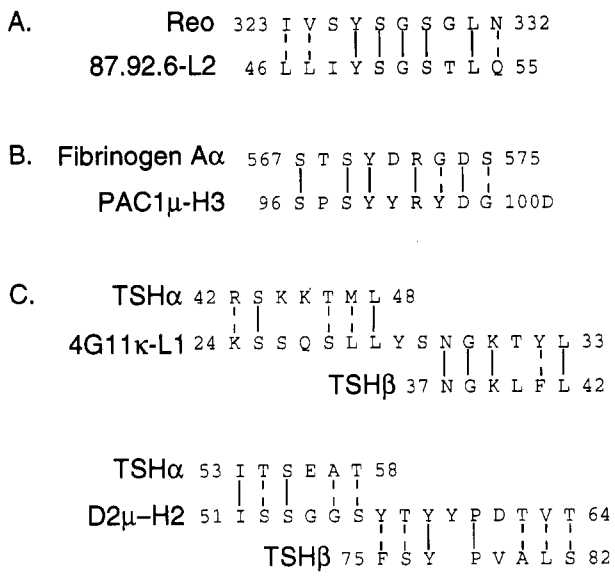

FIGURE 3: Alignment between ligand and relevant CDR regions in the reovirus, fibrinogen, and TSH systems. Amino acids are designated.

platelet fibrinogen receptor (GP IIb/IIIa). The binding of fibrinogen to the GP IIb/IIIa complex is mediated, in part, by an Arg-Gly-Asp (RGD) sequence within the fibrinogen A α-chain. The binding of PAC1 to the platelet fibrinogen receptor is inhibited by both fibrinogen and RGD-containing peptides. Like fibrinogen, PAC1 binds to GP IIb/IIIa only on activated platelets (Shattil et al., 1985). The binding of both PAC1 and fibrinogen is inhibited by RGD-containing peptides. Moreover, PAC1 and fibrinogen compete with each other for binding to GP IIb/IIIa (Shattil et al., 1986; Bennett et al., 1988). These data suggested that the antigenic site on GP IIb/IIIa for PAC1 is close to or identical with the binding site for fibrinogen.

Monoclonal antibodies, D2 and 4G11, which bind to the TSH receptor were selected by the autoantiidiotypic approach after injection with TSH. These antibodies were selected because their binding to thyroid membranes was inhibited by TSH, and conversely, they inhibited TSH binding. Moreover, they inhibited functional properties of TSH including stimulated mitogenesis and TSH-stimulated cAMP production (Hill et al., 1987). These findings suggested that D2 and 4G11 combined with a site at or near the TSH binding site on the TSH receptor.

Sequence Similarities between Ligand and CDRs of Anti-Receptor Monoclonal Antibodies. While the possibility was raised that structures within complementarity-determining regions (CDRs) from such anti-receptor monoclonal antibodies might mimic ligand (Gaulton et al., 1984), it is known that antigen binding is often mediated by more than one CDR (Laver et al., 1990; Rees, 1987; Bentley et al., 1990). However, the relative contribution of binding by any CDR for those antigen–antibody complexes that have been crystallographically resolved is not known. In fact, primary sequence similarities between regions of the ligand and anti-receptor monoclonal antibody CDRs have been identified (Bruck et al., 1986; Taub et al., 1989, 1992) and further characterized.

In the prototype reovirus system, Bruck et al. (1986) first determined the amino acid sequence of the variable regions of 87.92.6 and found a similarity with the reovirus hemagglutinin that comprised the 7/9 amino acids just proximal to the H2 region (later shown to be of less significance) and the 6/7 amino acids in the L2 region (Figure 3A). Subsequent studies in the fibrinogen/fibrinogen receptor system added additional evidence to the molecular mimicry hypothesis (Taub et al., 1989). A region in PAC1-H3 that had a 6/8 residue similarity with a RGD-containing region in the fibrinogen involved in fibrinogen receptor binding was observed (Figure 3B). In particular, an H3 RYD sequence that corresponds to the RGD sequence in fibrinogen was found. In the TSH/TSH receptor system, Taub et al. (1992) found 10/13 and 12/14 residues in two different autoantiidiotypic anti-TSH receptor antibodies to be similar to the TSH ligand (Figure 3C).

The criteria used for determining which amino acid segments within the six CDR regions in a given anti-receptor antibody might be involved in binding were based on sequence similarity between ligand and CDR regions, the likelihood that such a sequence would be in the β-turn region of the CDR, and the uniqueness of a particular CDR sequence. For instance, in 87.92.6, the regions of similarity between the hemagluttinin and L2 were both predicted to have β-turns and a significant degree of structural as well as sequence homology. In PAC1, 5/6 of the CDR regions were common to many antibodies, and only H3 had a large and unusual structure. In the TSH system, relevant CDRs were identified in which there was sequence similarity with nonoverlapping segments of both chains of TSH.

Sequence similarities between ligand and anti-receptor CDR antibodies are not found uniformly. Not all anti-receptor monoclonal antibodies interact directly with ligand binding sites in receptors. Instead, they may sterically hinder ligand binding and, therefore, would not be expected to mimic the ligand at the level of primary sequence. Often the regions of similarity between CDR regions and the ligand are limited as demonstrated in the reovirus, fibrinogen, and TSH analyses. These similarities can only be detected by careful alignment analyses. Alternatively, similarity could be based solely on secondary or tertiary structure and not on primary amino acid sequence. In addition, predictions of similarity based simply on sequence may not be borne out by structural or biochemical analyses. The significance of such sequence alignments is assessed through analyses of CDR-derived peptides in biochemical assays.

Proof of the Involvement of Ligand-Mimicking CDRs in the Interaction between Monoclonal Antibody and Receptor. Biochemical Analyses. Substantial documentation of the biological importance of the ligand-mimicking CDR regions has been obtained for the reovirus, fibrinogen, and TSH systems. In the reovirus system, synthetic peptides corresponding to the area of primary sequence similarity, L2, were found to bind to neutralizing mAb 9BG5 against which the mAb 87.92.6 is directed (Williams et al., 1988). The peptides competed with mAb 87.92.6 and reovirus type 3 for binding by mAb 9BG5 and prevented mAb 87.92.6 and reovirus type 3 from binding to the cell-surface reovirus type 3 receptor. Other properties of the MAb (87.92.6) were shared by L2-related peptides. Dimeric forms of L2 peptide reproduced several effects of 87.92.6 including inhibition of murine fibroblast growth, downmodulation of the reovirus receptor, alterations in Schwann cell growth, and changes in the growth and differentiation of oligodendrocytes (Williams et al., 1989, 1990, 1991a,b; Cohen et al., 1991; Saragovi & Greene, 1992). Placing alanine at specific positions within the L2 peptide demonstrated the importance of specific amino acid residues and structural features that mediate attachment (Williams et al., 1991b; Sauve et al., 1991). Most importantly, constraining the CDR peptide by precisely placed disulfide bonds enhanced the biological potency (Williams et al., 1991a).

In the fibrinogen–receptor system, a 21 amino acid synthetic peptide encompassing the H3 region inhibited fibrinogen-

dependent platelet aggregation as well as the binding of PAC1 ($K_i = 10\ \mu M$) and fibrinogen ($K_i = 5\ \mu M$) to activated platelets (Taub et al., 1989). The RYD region of H3 appeared to be central to its function, because substitution of the tyrosine with glycine increased the inhibitory potency of the peptide 10-fold, while replacing the tyrosine with D-alanine or inverting the RYD sequence sharply reduced the inhibitory potency. Thus, the linear sequence, RYD, within H3 of PAC1 mimicked the RGD receptor recognition sequence in fibrinogen.

In the TSH–receptor system, Taub et al. (1992) found that antibody-derived peptides 4G11-L1 and D2-H2 show sequence similarity to regions of TSHα and TSHβ that had been previously implicated in the interaction of the hormone with its receptor. They tested the inhibitory effects of synthetic peptides from D2-H2 and 4G11-L1 on the binding of the corresponding antibodies to rat thyroid FRTL-5 cells and found an EC_{50} of 0.1 and 1 μM, respectively. TSH-derived peptides with similarity to D2-H2 and 4G11K-L1 showed a significant but lesser effect on the binding of 4G11 or D2 to thyroid cells. Additionally, D2-H2 and 4G11-L1 inhibited TSH-stimulated cAMP production in FRTL-5 cells, D2-H2 most strongly (EC_{50} 10 mM). Thus, linear sequences from the CDRs of these autoantiidiotypic antibodies with similarity to sequences from both subunits of TSH appeared to interact with the TSH receptor.

Molecular Modeling of CDRs of Anti-Receptor Antibody and Ligand. Implications for Receptor–Ligand Interactions. The shared structure between reovirus type 3 polypeptide and anti-receptor antibody 87.92.6 defined the oligopeptide-neutralizing/cell-attachment epitope of reovirus type 3. Computer modeling of this epitope, by use of sequence similarities of known immunoglobulin CDR β-loop conformations, permitted an examination of the predicted secondary structure of this epitope (Williams et al., 1991b). Further peptide studies indicated that hydroxyl groups of several residues are involved in contacting the reovirus type 3 receptor, including Tyr^{49}, Ser^{50}, Ser^{52}, and Thr^{53} in the antiidiotypic sequence, corresponding to Tyr^{326}, Ser^{327}, Ser^{329}, and Ser^{325} in HA3, respectively. Only Ser^{50} of the antiidiotypic sequence, corresponding to Ser^{327} of HA3, significantly altered neutralizing antibody binding. The modeled structures, in combination with these functional studies, allowed the development of a model of the interaction of specific residues with sialic acid, which serves as a part of reovirus type 3 receptor. These models revealed that similar amino acid residues and side-chain geometries may be utilized by the reovirus type 3 and influenza hemagglutinins in their interactions with cell-surface receptors.

Initial analyses of the anti-fibrinogen receptor antibody, PAC1, implicated H3 as having a predominant role in the antibody–receptor interactions. The third CDR in the heavy chain of antibody PAC1 (PAC1-H3) is very large and unique due to the insertion of a novel D region segment. The RYD sequence within PAC1-H3 was predicted to be in the proper conformation to behave like the RGD sequence in fibrinogen, and biochemical analyses confirmed these predictions (Taub et al., 1989). More recently, other antibodies to the fibrinogen receptor as well as unrelated antibodies have been found to contain the RYD motif. Modeling has suggested that the configuration of the particular RYD motif is important in determining receptor specificity (Tomiyama et al., 1992). As further confirmation of the importance of PAC1-H3, antibodies directed against PAC1-H3 derivative peptides behave like antiidiotypes in binding to PAC1 and to fibrinogen (Abrams et al., 1992). These antiidiotypic, anti-fibrinogen antibodies have been used to identify the fibrinogen receptor contact sites within fibrinogen.

The data on the TSH receptor monoclonal antibodies support previous studies, indicating the complexity of the interaction between TSH and its receptor. It is also intriguing that the TSHα sequences predicted by the alignments with D2 and 4G11 match sequences in the common α-subunit of hCG, predicted by a computer-assisted three-dimensional model to be at the interface of binding between hCG and its receptor. Moreover, hCGβ sequences homologous to the TSHβ sequences are predicted by the model to be at the same interface (Lustbader & Canfield, 1990). However, in the computer model, not all domains at the interface are presented by these four peptides. Consistent with this observation and the hypothesis that the biological activation domain of TSH may not completely overlap with the receptor binding domain (Kohn et al., 1986; Kasuga et al., 1991; Nagayam et al., 1991), neither D2 nor 4G11 increases cAMP levels. As a result, it is unlikely that when bound to receptor, these antibodies imitate all facets of the receptor–TSH interaction.

Therapeutic Implications. Designer Mimetics. The immunologic approach described here is useful for studying the structural basis of receptor–ligand interactions. In addition, these studies have therapeutic implications because peptide and nonpeptide mimetics can be developed that have increased affinity for the relevant receptor. Such molecules could be useful for certain types of therapeutic interventions. For example, agents blocking the fibrinogen receptor could be useful in treating hypercoagulation states and preventing myocardial infarctions. Agents that block the TSH receptor could be useful in treating thyroid disease.

Thus far, in the reovirus system, both peptides and nonpeptides related to L2 of the anti-receptor antibody, 87.92.6, have been developed that demonstrate appreciable affinity for the reovirus receptor. Peptides derived from antibody hypervariable region sequences can bind antigens with similar specificity, but with 2–3-order lower affinity than that of the intact antibody or ligand. To improve the affinity, cyclic and dimeric peptide analogs of L2 of 87.92.6 were developed (Williams et al., 1991a; Saragovi & Greene, 1992). The cyclic peptides were utilized to prove the optimal conformation for binding to both the receptor and anti-ligand antibody (9B.G5). By dimerization or constraint of the conformation of these peptides, higher affinity binding was produced. By utilization several different cyclic peptides, the optimal conformation for binding was established. The conformationally optimized cyclic peptide possessed >40-fold higher affinity for the receptor than for the linear analog.

When considering the development of therapeutics, peptides have several disadvantages including insolubility, immunogenicity, and instability. The benefit of using a monoclonal antibody CDR as a basis for designing nonpeptidyl mimetics is that the structure of the β-loop within the CDR can be more accurately predicted than the corresponding region of the ligand. A technique for producing nonpeptide compounds (mimetics) of designed specificities was developed that permitted the synthesis of a conformationally restricted β-loop structure that is an analog of L2. Like L2, this mimetic mimicked the binding and functional properties of 87.92.6 (Saragovi et al., 1991). Binding of either 87.92.6, peptide analogs, or the 87.1 mimetic to the cellular receptor inhibited cellular proliferation.

Such studies may lead to strategies for the synthetic design of antibody-complementarity regions, ligands, and other pharmacologically active nonpeptidyl agents. In fact, the potential utilization of similar mimetics extends beyond the receptor–ligand systems described here. In the past, anti-

viral and anti-tumor antiidiotypic antibody therapies have been devised [Nisonoff & Lamoyi, 1981; Campbell et al., 1988; Kang et al., 1992; for reviews, see Kohler et al. (1989), Bhattacharya et al. (1991), and Nissonoff (1991)]. For example, Kohler and colleagues have shown that antiidiotypic antibodies can be isolated that are directed against a primary antibody to a T-cell leukemia antigen. Such antiidiotypic antibodies which mimic the tumor antigen can be prepared in much larger quantity than the original tumor antigen and can be used to directly immunogenize the host against the tumor. By analogy with the reovirus mimetic, either nonpeptidyl or peptidyl mimetics of the essential CDR regions within such antiidiotypic antibodies could be synthesized and modified to make them immunogenic. They could be used in the host to generate an immune response to the tumor. Use of such mimetic derivatives would prevent the development of unwanted immune responses directed against most portions of an injected antiidiotypic antibody molecule. Another recent application of the mimetic concept was the design and synthesis of a CD4 β-turn mimetic that inhibits human immunodeficiency virus viral envelope glycoprotein gp120 binding and infection of human lymphocytes (Chen et al., 1992). In this example, the mimetic was to the complementarity determining 2-like region of CD4. Thus, nonpeptide mimetics may have a broad application in rational drug design.

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