Inhibition of Self-Binding Antibodies (Autobodies) by a V_H-Derived Peptide

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The self-binding properties of a dominant idiotypic antibody (T15) and a minor idiotypic antibody (M603), both specific for phosphorylcholine, were examined as models of self-binding antibodies (autobodies). Observed differences in the self-binding affinity of T15 and M603 relate to variable sequence differences in their respective heavy and light chains. A molecular recognition theory based on the translation of coding and noncoding DNA strands was used to identify complementary amino acid sequences responsible for self-binding. The second hypervariable region of the heavy chain domain, extending into the third framework region, was predicted as the primary self-binding locus. Among peptides synthesized with different variable heavy and light chain regions, a 24-residue peptide spanning the second hypervariable and third framework regions of the heavy chain of T15 was nearly as effective as phosphorylcholine in inhibiting the self-binding complexes.

ANTIBODIES, MEMBERS OF THE IMmunoglobulin (Ig) supergene family, are evolutionarily related and functionally connected to form the basis of the immune network (*1*). The idiotypic/anti-idiotypic relations that regulate the immune network have been extended to include Igs that express complementary paratopes and idiotopes. These antibodies have been called autobodies to specify the self-binding activity (*2, 3*). Immunoglobulins of the TEPC 15 (T15) idiotype exhibit the peculiar property of binding to themselves (*2*). T15 is the prototype of a mouse antibody family specific for the epitope phosphorylcholine (PC), which is an immunodominant determinant of the C-polysaccharide found on a number of pathogenic and nonpathogenic bacteria and on some worms. T15 self-binding or aggregation can easily be demonstrated by solid-phase binding assays (*2, 3*). The T15-T15 binding is highly dependent on the available valency of the binding antibody, in that dimeric T15 binds more strongly than monomeric T15 and pentameric T15 IgM binds more strongly than the T15 dimer (*3*). The specificity of T15 self-binding is further exemplified by the inhibition of these complexes with PC and PC analogs (*3*). Anti-idiotypic antibodies have been raised that group antibodies to PC into the T15, the M167, and the M603 idiotype families (*4*). The heavy chain of the PC-specific antibodies in BALB/c mice is encoded by a single gene of the heavy chain variable region family (V_H) (*5*). However, antibodies to PC have entirely different light chains (V_L), which are encoded either from the Vκ8 (M603), Vκ22 (T15), or Vκ24 (M167) light chain subgroups. Minor differences in the heavy and light chains result from point mutations, which may affect idiotype expression (*6*) or PC binding specificity (*7*).

In an earlier examination of the binding of T15 to other anti-PC antibodies representative of the idiotype families (*2, 3*), we observed that T15 could bind to insolubilized M603 but not to insolubilized M167. We tested the binding of ^{125}I-labeled M603 and T15 dimer to a panel of insolubilized Igs. M603 binds more strongly to T15 than to itself, whereas only trace amounts of binding to M167 could be measured (Table 1). The specificity of M603 and T15 binding is established by complete inhibition with PC. Labeled M603 and T15 do not bind to M315, a control IgA that has specificity for trinitrophenyl.

Since radiolabeled T15 binds more strongly to T15 than to M603 and radiolabeled M603 binds more strongly to T15 than to M603 (Table 1), the data establish that the affinity of T15 self-binding seems to be higher than that of M603 self-binding. In addition, the bindings of radiolabeled T15 to insolubilized T15 and radiolabeled M603

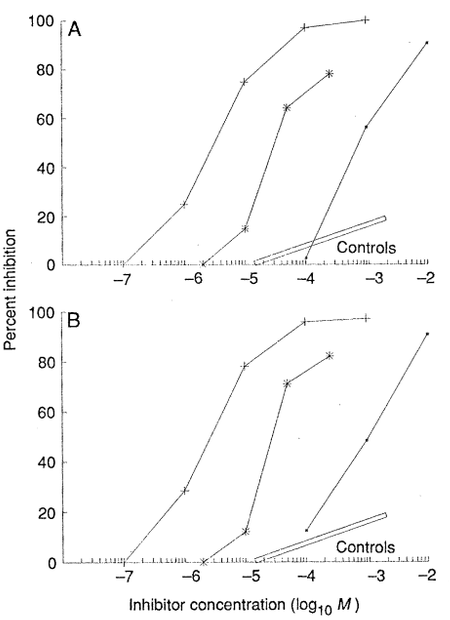

Fig. 1. Inhibition of (**A**) T15 and (**B**) M603 self-binding by PC, choline, and peptides. The experiment was performed in a manner similar to that described in Table 1. ^{125}I-labeled T15 was added to T15-coated plates (A) or ^{125}I-labeled M603 was added to M603-coated plates (B) in the absence or the presence of inhibitors. The inhibitors used were PC (+), choline (■), and peptide T15H(50–73) (*). Controls were peptide T15H(50–61), peptide T15H(63–73), a mixture of peptide T15H(50–61) and T15H(63–73), and peptides T15L(24–40), 4C11H(84–91), 37A4H(92–111), and 37A4L(89–106). Numbers in parentheses represent positions of amino acid residues corresponding to heavy and light chains of the antibodies (*16*). 4C11 is an anti-idiotypic antibody to T15 (*14*), and 37A4 is an antibody to alprenolol (*17*). The peptides were synthesized by solid-phase methods (*18*) and purified by high-performance liquid chromatography on a C_{18} column. After incubation for 18 hours the wells were washed, and the amount of ^{125}I bound in individual wells was determined. The percent inhibition of binding is plotted against the concentration of inhibitors.

Table 1. Specificity of binding of M603 and T15. Microtiter plates were coated for 18 hours with 0.1 ml of purified dimeric antibodies (5 μg/ml) in phosphate-buffered saline (PBS), washed three times, and incubated for 2 hours with 1% bovine serum albumin (BSA) in PBS. After the plates were washed, 0.1 ml of ^{125}I-labeled dimeric M603 (25,000 cpm) or T15 (25,000 cpm) in 1% BSA and 0.05% Tween-20 in borate-buffered saline was added and incubated for 18 hours in the absence or presence of PC ($10^{-3}M$). After incubation, the wells were washed and the amount of radioactive material bound (counts per minute) was determined in a gamma counter. Binding is expressed as mean ± SD of experiments repeated four times. The amount of positive binding is significantly different in each assay at the >99% confidence level.

Plates coated with	^{125}I-labeled M603 binding		^{125}I-labeled T15 binding	
	Without PC	With PC	Without PC	With PC
T15	3132 ± 131	160 ± 12	6025 ± 203	157 ± 13
M603	2195 ± 137	151 ± 11	4775 ± 157	155 ± 9
M167	276 ± 12	140 ± 9	387 ± 21	145 ± 7
M315	136 ± 4	132 ± 5	145 ± 7	147 ± 6

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Table 2. Comparison of self-binding of M603 and T15. Binding inhibition assays were performed as in Table 1. All antibodies used were dimers. The amount required for 50% inhibition was extrapolated from an inhibition titration curve. Each experiment has been repeated three times. Fifty percent inhibition is expressed as mean ± SD (n = 3) and is significantly different in each inhibition assay at the >99% confidence level.

Unlabeled antibody inhibitor	Amount of antibody (μg/ml) required for 50% inhibition in	
	T15-T15 binding	M603-M603 binding
T15	0.15 ± 0.02	0.08 ± 0.01
M603	8.8 ± 0.7	1.7 ± 0.2

to insolubilized M603 were inhibited by unlabeled T15 and M603, respectively. The concentrations required for 50% inhibition are shown in Table 2. T15 is the more efficient inhibitor in both cases. These data confirm the different strengths of complexing in T15 and M603 and indicate that their distinctive self-binding capacities are related to differences in their primary structures.

The primary structures of T15 and M603 were examined for hydropathic complementarity of amino acids in an effort to identify the critical sequence region involved in self-binding. There is a significant correlation between the hydropathic scores of amino acids encoded by a DNA coding strand and the hydropathic scores of those of the noncoding strand (*8*). Thus, sequence regions encoded by complementary DNA strands might form amphiphilic type structures that bind to one another. Sequence regions involved in protein and peptide interactions have been identified by this approach and have been found to bind to each other in solution and in solid phase (*8, 9*); antibodies to such peptides have exhibited anti-idiotypic properties (*10*).

Computer searches for sequence (hydropathic) complementarity were performed in the V_H and V_L of M603 and in the T15 variable domains. The most significant domains of sequence complementarity for all cases were the second complementarity-determining region of V_H (CDR2) extending into the third framework region (FR3). These domains, which are predicted to be of general importance in defining idiotypic expression (*11*), correspond to various idiotopes as defined by sequence analysis and idiotope mapping by antibodies to peptides and more recently by electron microscopy of anti-idiotypic antibodies (*12*). These domains have been referred to as idiotope-determining regions (IDR) (*11*).

The complementary hydropathic sequences of T15 and M603 are shown in Table 3. Residues 50 to 60 of T15 are complementary to residues 63 to 74 (Table 3A), nine amino acids of one segment being complemented in sequence order by nine amino acids in the other segment. The alanine residue at position 55 is complementary to both glycine and arginine at positions 68 and 69, respectively. Two alignments are observed for sequence complementarity between T15 and M603 (Table 3B). One alignment is identical to that for T15-T15. The other occurs from the sequence changes in M603 ($Ala^{55} \rightarrow$ Gly and $Asp^{57} \rightarrow$ Lys), reducing the match to seven complementarities within the segments. The self-complementarity within the M603 sequence is shown in Table 3C. Two alignments, each with seven complementary pairs, are observed. Within the set of T15 and M603 antibodies, the rank order of pairwise binding correlates with the number of residue changes [T15-T15 (0) > T15-M603 (2) > M603-M603 (4)] in the proposed binding site and also correlates with the maximum possible number of complementary amino acid pairs [T15-T15 (18) > T15-M603 (16) > M603-M603 (14)]. It follows from these results that the predictive method for identifying sequence complementarity in T15 and M603 concurs with the experimentally observed gradient in T15 and M603 complexing.

To test the predicted role of CDR2 and FR3 of V_H in defining the binding site loci of T15 and M603 self-complexes, we synthesized peptides spanning residues 50 to 73 in T15 and assayed for their ability to inhibit complex binding (Fig. 1). To compare the inhibitory capacity of the peptides, we included choline and PC as positive controls. Only one peptide, T15H(50–73), corresponding to residues 50 to 73 of T15 heavy chain, inhibits both T15 (Fig. 1A) and M603 (Fig. 1B) self-binding. This peptide is a more effective inhibitor than choline over the range of inhibitor used, although it is slightly less effective than PC. Peptides T15H(50–61) and T15H(63–73) did not show any ability to inhibit the self-binding, either individually or when pooled, indicating that the inhibitory effect is specific to a conformational property of peptide T15H(50–73). No inhibition was observed with control peptides T15L(24–40), 4C11H(84–91), 37A4H(92–111), and 37A4L(89–106). Thus, the inhibitory capacity of peptide T15H(50–73) is in agreement with the data obtained by complementary sequence analysis. While these results do not rule out the existence of other inhibitory peptides derived from different sequence regions of V_H or V_L, the high inhibitory efficiency of the T15H(50–73) peptide indicates that the heavy chain region spanning residues 50 to 73 participates in the self-binding site. Furthermore, peptide

Table 3. Structural complementarities for T15 and M603 complexes. Vertical bars denote amino acid complementarity. Boxes indicate residues differing from the T15 sequence at the corresponding position. Complementary amino acid pairs were determined from the conventional (5′ to 3′) translations of amino acid codons and their complements. A match (complementarity) is indicated when there exists a codon for an amino acid in one sequence whose complement translated 5′ to 3′ gives the amino acid in the second sequence (*8*). Sequences were taken from data in (*16*).

A) T15-T15	50 60	
	A S R N K A N D Y T T	T15 VH CDR-2
	S A S V K GR F I V S R	T15 VH CDR-2
	63 74	
	A S R N K A N D Y T T	T15 VH CDR-2
	S A S V K GR F I V S R	T15 VH CDR-2
B) T15-M603	A S R N K A N D Y T T	T15 VH CDR-2
	S A S V K GR F I V S R	M603 VH CDR-2
	A S R N K [G] N [K] Y T T	M603 VH CDR-2
	S A S V K GR F I V S R	T15 VH CDR-2
C) M603-M603	59 66	
	T T E Y S A S V	M603 VH CDR-2
	G R F I V S R D	M603 VH CDR-2
	68 75	
	A S R N K [G] N [K] Y T T	M603 VH CDR-2
	S A S V K GR F I V S R	M603 VH CDR-2

T15H(50–73) is an efficient inhibitor of the binding of labeled T15 to insolubilized PC antigen (*13*). However, two monoclonal anti-idiotypic antibodies to T15 (*6, 14*) do not bind to the peptide T15(50–73) (*13*).

The self-binding of the T15-M603 family demonstrates a novel property of the Ig family. A functional site that exhibits self-complementarity and can induce self-binding was identified in the V_H chain region. The self-binding site is proximal to the antigen-binding site, since the specific hapten PC effectively competes with T15 binding. The self-binding site should be viewed in the wider context of the biological properties of molecules in the Ig supergene family (*15*). Members of this family are integral membrane proteins that play different roles in the immune system. Major histocompatibility complex (MHC) proteins and T cell receptors are different from Igs because they have a second active site in addition to the antigen-binding site. The second site is designed to interact with proteins (MHC molecules) encoded by so-called immune response genes. Since the architecture of MHC molecules and T cell receptors is similar to the structure of Igs, it should not be surprising to detect a self-binding property on the T15 antibody that is not obviously related to its antigen specificity. The analogy to the immune response site on T cell receptors extends further into the character of the self-binding site of T15, which appears to be of low affinity and dependent on quaternary interactions such as intermolecular valencies. One might speculate that the T15 self-binding site is related to the intermolecular recognition site in non-antibody molecules in the Ig supergene family. The proximity of the antigen-binding site and the self-binding site in T15 seems to be another feature shared with the dual recognition specificity of T cell receptors.

The biological function of a self-binding antibody within the idiotypic network remains to be elucidated. As the self-binding site is functionally related to the antigen-binding site, a simple feedback mechanism in the immune response can be envisioned. Such a model could be extended to the role of B cells as antigen-presenting cells whereby circulating antibody could block antigen presentation to T cells. The biological relevance of self-binding antibodies would be strengthened if it could be demonstrated with other antibodies.

REFERENCES AND NOTES

1. N. K. Jerne, *Ann. Immunol. (Paris)* **125C**, 373 (1974).
2. C.-Y. Kang and H. Kohler, *J. Exp. Med.* **163**, 787 (1986); *Ann. N.Y. Acad. Sci.* **405**, 114 (1986).
3. C.-Y. Kang, H. L. Cheng, S. Rudikoff, H. Kohler, *J. Exp. Med.* **165**, 1332 (1987).
4. M. Potter and R. Liberman, *ibid.* **132**, 737 (1970).
5. R. M. Perlmutter *et al.*, *Adv. Immunol.* **35**, 1 (1984).
6. M. K. Wittner, M. A. Bach, H. Kohler, *J. Immunol.* **128**, 595 (1982).
7. B. Diamond and M. D. Scharff, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 5841 (1984).
8. J. E. Blalock and E. M. Smith, *Biochem. Biophys. Res. Commun.* **121**, 203 (1984); J. E. Blalock and K. L. Bost, *Biochem. J.* **234**, 679 (1986); K. L. Bost and J. E. Blalock, *Mol. Cell. Endocrinol.* **44**, 1 (1986); D. J. J. Carr, K. L. Bost, J. E. Blalock, *J. Neuroimmunol.* **12**, 329 (1986); J. J. Mulchahey, J. D. Neill, L. D. Dion, K. L. Bost, J. E. Blalock, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 9714 (1986); T. J. Gorcs, P. E. Gottschall, D. H. Coy, A. Arimura, *Peptides* **7**, 1137 (1986); K. L. Bost, E. M. Smith, J. E. Blalock, *Biochem. Biophys. Res. Commun.* **128**, 1373 (1985).
9. Y. Shai, M. Flashner, I. M. Chaiken, *Biochemistry* **26**, 669 (1987).
10. L. R. Smith, K. L. Bost, J. E. Blalock, *J. Immunol.* **138**, 7 (1987).
11. T. Kieber-Emmons and H. Kohler, *Immunol. Rev.* **90**, 29 (1986); T. Kieber-Emmons, E. Getzoff, H. Kohler, *Int. Rev. Immunol.* **2**, 339 (1987).
12. K. H. Roux, W. J. Monafo, J. M. Davie, N. S. Greenspan, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 4984 (1987).
13. The assay plate was coated with 0.2 μg of PC-BSA for 18 hours, then washed and blocked by 1% BSA solution. Radiolabeled T15 was then incubated in the assay plate in the absence or the presence of T15H(50–73). The assay plate was coated with 1 μg of T15H(50–73) and keyhole limpet hemocyanin (KLH) for 18 hours, then washed and blocked by 1% BSA solution. After the assay plate was washed, anti-idiotypic antibodies to T15 (4C11 and F6-3) were added. Enzyme-coupled goat antibody to mouse Ig was used for detecting antibody binding to the assay plate.
14. H.-L. Cheng *et al.*, *Mol. Immunol.* **25**, 33 (1988).
15. L. Hood, J. M. Kronenberg, T. Hunkapiller, *Cell* **40**, 225 (1985).
16. E. A. Kabat, T. T. Wu, H. Bilosfsky, M. Reid-Miller, H. Perry, *Sequences of Proteins of Immunological Interest* (Department of Health and Human Services, Washington, DC, 1983).
17. C. Nahmias, D. Strosberg, L. J. Emorine, *J. Immunol.* **140**, 1034 (1988).
18. R. Merrifield, *J. Am. Chem. Soc.* **85**, 2149 (1963).

19. Supported by grant AG04180 (H.K.), the Center for Applied Molecular Biology and Immunology at the State University of New York at Buffalo (T.K.E.), and the Health Instruments and Devices Institute at the State University of New York at Buffalo (H.K.). We thank P. D. Hoeprich and M. Tischler for the synthetic peptides from the T15 and 4C11 sequence, D. Capra for peptides from the 37A4 sequence, and A. Bruce for assistance in the processing of this manuscript.

24 September 1987; accepted 24 March 1988

Aldolase Activity of a *Plasmodium falciparum* Protein with Protective Properties

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Immunization with a 41-kilodalton blood stage antigen (p41) of *Plasmodium falciparum* induces immunity to malaria in monkeys. However, antigenic polymorphism and repetitive amino acids commonly found in protective antigens complicate vaccine development. The gene encoding p41 has now been cloned and analyzed. Sequencing and hybridization studies revealed that the gene structure is highly conserved in 14 parasite isolates from three continents. This finding and the lack of repetitive amino acids in the translated DNA sequence may indicate that p41 has an essential function. In this study the protein was found to be 60 percent homologous to the key glycolytic enzyme aldolase from vertebrates, and the affinity-purified p41 protein from parasites showed aldolase activity.

IMMUNIZATION OF MONKEYS WITH IRradiated merozoites and schizonts of the human malaria parasite *Plasmodium falciparum* induces a marked degree of protection against challenge with viable parasites (*1, 2*). Purified proteins of these blood stages of the parasite induce a similar degree of protection (*3, 4*). Among these antigens is a 41-kD polypeptide termed p41 that is expressed preferentially at the end of maturation in the asexual blood stage of the parasite. It is detected in association with membrane preparations of parasitized red blood cells and is found in increased concentrations in polar secretory organelles or rhoptries of the parasite (*5, 6*). Antibodies to the 41-kD rhoptry component react with a large number of *P. falciparum* isolates by immunofluorescence, suggesting that at least parts of the sequence are common in these isolates (*5, 7*). A malaria vaccine based on p41 might thus be active against a large number of parasite variants; we therefore analyzed the gene encoding p41.

We constructed λGT11 phage expression libraries with randomly sheared genomic

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