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Mechanism and Functional Role of Antibody Catalysis

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ABSTRACT

The light (L) chain of a model antibody (Ab) was deduced to contain a serine protease-like catalytic site capable of cleaving peptide bonds. The catalytic site is encoded by a germline V_L gene. The catalytic activity can potentially be improved by somatic sequence diversification and pairing of the L chain with the appropriate heavy chain. Autoimmune disease is associated with increased synthesis of antigen (Ag)-specific Abs, but the reasons for this phenomenon are not known. Only recently has attention turned to the functional role of the catalytic function. Preliminary studies confirm that the catalytic cleavage of peptide bonds is a more potent means to achieve Ag neutralization, compared to reversible Ag binding. Administration of a monoclonal Ab to VIP in experimental animals induces an inflammatory response in the airways, suggesting that catalytic autoantibodies to this peptide found in airway disease and lupus are capable of causing airway dysfunction. The phenomenon of autoantibody catalysis can potentially be applied to isolate efficient catalysts directed against tumor or microbial Ags by exposing the autoimmune repertoire to such Ags or their analogs capable of recruiting the germline V_L gene encoding the catalytic site.

Index Entries: Catalytic antibodies; light chain; germline genes; somatic maturation; VIP; asthma.

INTRODUCTION

Certain antibodies (Abs) combine the ability to bind ligands and to catalyze their chemical transformation. Presumably, the development of catalytic activity in Abs occurs by means similar to those in enzyme evolution, i.e., sequence diversification guided by the biological advantages associated with acquisition of the catalytic function. Catalysts have been identified in subpopulations of Abs found in patients with autoimmune disease *(1–6)* and in undiseased humans subjects *(7,8)* and animals *(8)*; Abs raised by immunization with the substrate–carrier conjugates *(9–13)* and substrate analog–carrier conjugates *(14–16)*, anti-idiotypic Abs to enzymes *(17,18)*; Abs to transition-state analogs (TSA) *(19)*; and Ab light (L) chains in patients with respiratory disease *(20,21)* and multiple myeloma *(22–24;* Fig. 1).

This review collates evidence indicating that efficient catalytic Abs exist; autoimmune disease is associated with the synthesis of Abs specialized to recognize autoantigens; the biological efficacy of catalytic Abs is quantitatively greater and qualitatively different than their noncatalytic counterparts; various functional roles for catalytic Abs in innate and adaptive immunity can be posited on theoretical grounds, which, if validated experimentally, may explain certain aspects of self-tolerance and the destructive responses in autoimmune diseases.

BINDING AND CATALYSIS AS EFFECTOR FUNCTIONS

Interactions that lead only to the binding of the reactants and those that go on to effect the chemical transformation of one or more reactants have very different biological outcomes. In the case of an interaction limited to binding, one of the reactants serves as the receptor, acting as a switch to transduce the binding event into a signal recognizable by other proteins. For instance, the binding of an antigen (Ag) by an Ab expressed as a receptor on the B-cell surface can serve as the signal to drive the cells into clonal proliferation, and the binding of an Ag to a soluble Ab can promote the interaction of complement components with the Fc segment of the Abs leading eventually to activation of the complement cascade. Molecular interactions resulting in chemical catalysis, on the other hand, serve roles that include, but are not limited to, the signal transducing function. The two defining features of a catalyst are that it must transform the substrate (i.e., the ligand, the antigen) chemically; and it must turn over, i.e., a single catalyst molecule must be capable of being reused for the chemical transformation of multiple substrate molecules.

The biological functions of a protein Ag can potentially be irreversibly altered by a catalytic Ab. In the case of cleavage of peptide bonds

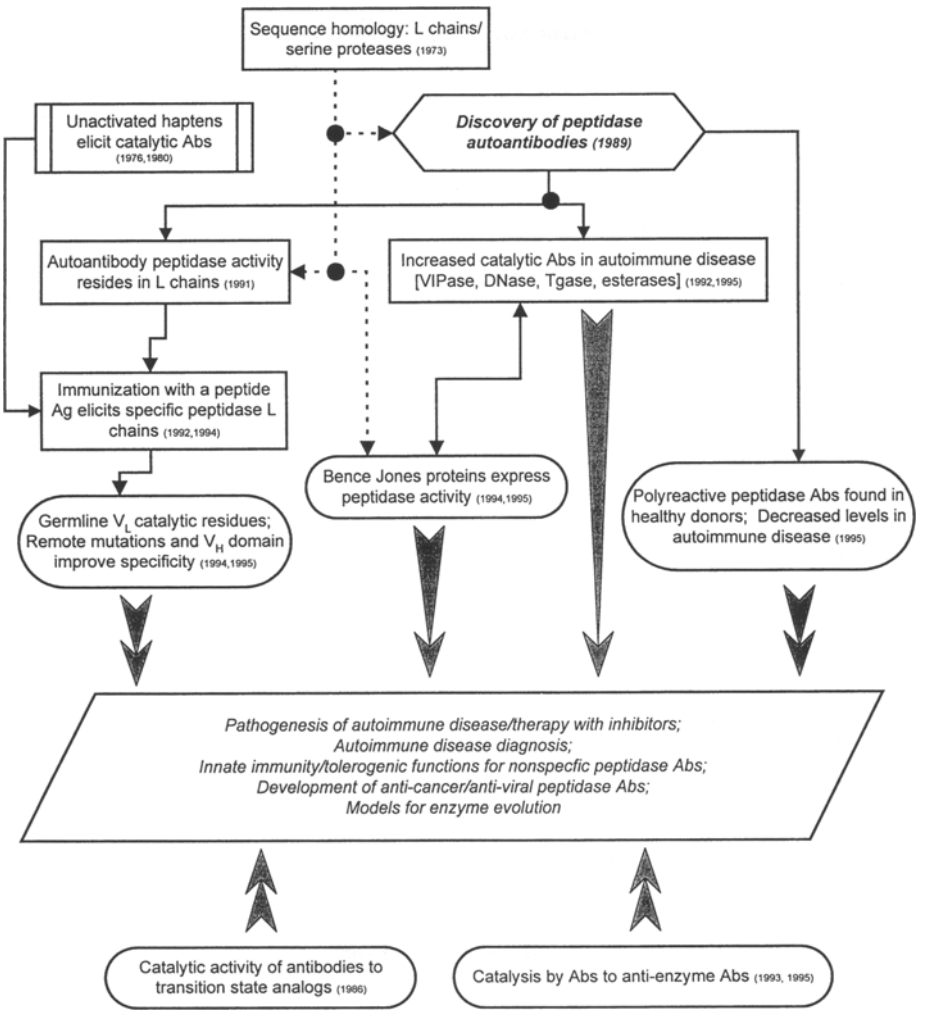

Fig. 1. Overview of the field of catalytic Abs. Year of publication shown in parentheses. Arrows trace links between the experimental developments and their implications. See text for references.

in a protein Ag by a catalytic Ab, the function of the protein may be lost permanently, or a new function may be acquired upon its fragmentation. Cleavage of the HIV-1 protein gp120 at certain peptide bonds, for instance, inactivates the infective capability of HIV-1 *(25)*, and cleavage of the neuropeptide substance P at certain bonds can generate smaller peptides with a biological activity profile different than that of the parent peptide *(26)*. The turnover capability implies that, on a molar basis, a catalytic Ab will be biologically more potent than a noncatalytic Ab that binds the Ag stoichiometrically. In the latter case, dissociation of the Ag from the Ab

restores its biological activity, and only one Ag molecule can be neutralized per Ag binding site expressed by the Ab.

ORIGIN AND MECHANISM OF CATALYTIC ACTIVITY

Biochemical and structural characterization of Abs carried out by the author and other groups support the following hypotheses: A germline-encoded peptidase activity resides in the V_L domain. The magnitude and quality of the peptidase activity encoded by the V_L domain is susceptible to maturation by somatic means, i.e., mutation and pairing with V_H domains. Preferential utilization of this germline activity and/or dysfunctional somatic diversification mechanisms lead to increased synthesis of catalytic Abs in autoimmune disease.

The L chain of an Ab raised to vasoactive intestinal polypeptide (VIP) displays the ability to cleave this peptide. Molecular modeling of the VIPase L chain showed that its Ser27a, His 93, and Asp1 residues (Kabat numbering) can be precisely superimposed on the corresponding active site Ser, His, and Asp residues of a non-Ab serine protease, subtilisin *(27)*. Mutants containing an Ala residue, instead of the wild-type Ser27a, His93, or Asp1, displayed loss of the peptidase activity, indicating that these residues constitute the catalytic site *(28)*. The VIPase V_L domain contains four amino acid replacements, compared to its germline V_L (GenBank number Z72384) and Jk1 gene counterparts, of which three are located in complementary deforming region (CDR)1 (His27d:Asp, Thr28e:Ser, and Ile34: Asn[germline-encoded residues shown second]) and one at the junction of the V_L and J segments (Gln96:Trp) (Fig. 2). Even though the three catalytic residues in the VIPase V_L domain (Ser27a, His93, Asp1) are present in the germline V_L gene, it could be argued that conformational changes induced by somatic mutations at remote sites are essential for the catalytic activity. To study this possibility, the germline configuration protein of the VIPase L chain was constructed by introducing the required four mutations, as described previously *(28)*. The purified germline L chain expressed catalytic activity at about 3.5-fold lower level than the mature L chain, as detected by cleavage of the synthetic protease substrate Pro-Phe-Arg-MCA (Gololobov and Paul, unpublished). Therefore, the somatic mutations in the V domain are not obligatory for expression of the catalytic activity.

Replacement of the components of the catalytic triad by mutagenesis was without effect on the affinity of the L chain for VIP, as indicated by near-equivalent K_m values of the wild-type and catalytically deficient mutants *(28)*. This observation can be understood from the transition state theory, which holds that catalyst binding to the unstable reaction intermediate between the reactant and the products, the transition state, reduces the activation energy and accelerates the reaction *(29)*. In the case of the L

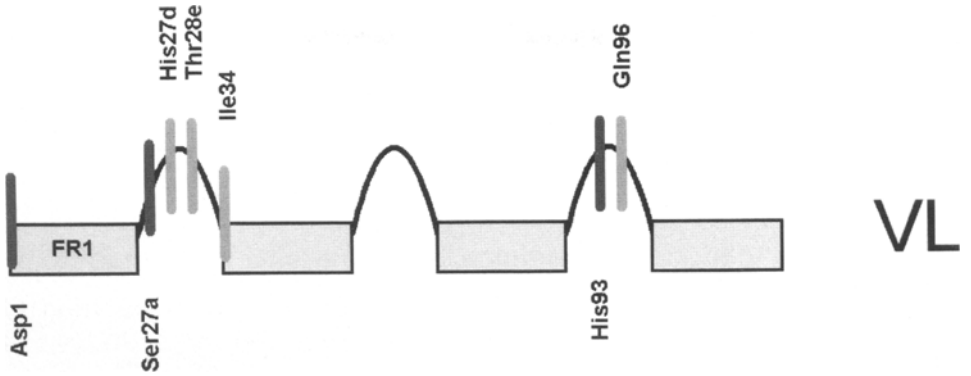

Fig. 2. Catalytic residues in anti-VIP chain (clone c23.5) shown as dark vertical lines. The V_L domain differs in its amino acid sequence from its germline counterpart in four positions (shown as grey vertical lines). The three catalytic residues are germline encoded.

chain, the catalytic residues are important in binding the VIP transition state, but not the stable state of the peptide. These observations have suggested that the catalytic subsite is physically and functionally distinct from the conventional Ag binding site.

Additional support for the central argument advanced above, i.e., a conserved, germline-encoded site is responsible for the catalysis, can be adduced from observations of polyreactive catalytic Abs *(30)*. These Abs recognize small synthetic peptides with low affinity, cleave peptides with quite different sequences, and are present in unimmunized donors, which suggest an unspecialized nature. This behavior is reminiscent of polyreactive Ag binding by certain Abs *(30)*, which are thought to be synthesized by $CD5^+$ B-cells *(31)*, contain germline or minimally mutated V regions *(32)*, and can bind structurally different Ags with low affinity *(33)*.

The germline origin of the catalytic subsite, although suggesting that the catalytic function is an expression of innate immunity, does not exclude a possible improvement in this function over the course of adaptive Ab responses. Polyclonal catalytic Abs isolated from patients with autoimmune disease display high affinities for their autoantigens, i.e., VIP *(1,2)*, thyroglobulin *(4)*, and DNA *(5,6)*, which is the classical sign that the Abs have been subjected to somatic mutations and clonal selection. Further, two recombinant L chains with efficient VIP-cleaving activities have been found to be extensively mutated, compared to their germline V_L gene counterparts *(21)*.

Pairing of the catalytic V_L domain with the appropriate V_H domain constitutes a second somatic process by which the activity of the germline site can be modulated. This is suggested by observations of improved catalysis by linking the model VIPase V_L domain with its natural V_H domain partner, but not with an irrelevant V_H domain *(34)*. Such improve-

ments could potentially be mediated by allosteric effects that improve the spatial position of the catalytic site for transition-state binding, or by introduction of new, chemically reactive amino acids in the catalytic subsite that participate directly in the mechanism of catalysis.

CATALYTIC ACTIVITY IN AUTOIMMUNE DISEASE

VIP-Cleaving Antibodies in Asthma

VIP-binding autoantibodies were observed in a subpopulation of patients with asthma *(35)*. Some of these Abs catalyzed the hydrolysis of VIP *(2,36)*. The activity was characterized as being caused by Abs, according to several criteria, including electrophoretic purity of the IgG, binding of the activity by immobilized protein G, a bacterial IgG-binding protein that binds the Fc region, and by antihuman IgG, and by the presence of the activity in Fab fragments. The VIP-hydrolyzing activity of IgG purified from 19 nonasthmatics and 33 asthmatic subjects was compared. Approximately 50% of IgG samples from asthmatic subjects displayed VIP-hydrolyzing activity $>$ mean $\pm$ 2 SD for IgG samples from the nonasthmatic controls ($P < 0.001$, Mann-Whitney U-test). The strong binding affinities (K_d, K_m) are typical of Abs. Fragments of VIP produced by the Abs were separated by reversed-phase high-pressure liquid chromatography (HPLC) and identified by N-terminal sequencing. Seven Ab-sensitive peptide bonds were identified *(2)*, of which six were clustered between residues 14–22. The epitope specificity of an Ab that cleaved a single bond in VIP [Gln(16)-Met(17)] was determined using synthetic fragments corresponding to linear, overlapping subsequences of VIP *(37)*. VIP[15–28], a large C-terminal subsequence, was deduced to be the Ab-reactive epitope, because it was bound with high affinity. A shorter subsequence, VIP[22–28] was bound by the Ab with low affinity, and inhibited the hydrolysis of VIP competitively. VIP[22–28], which is located four residues distant from the cleavage site, is evidently a subepitope that participates in Ab binding.

Thyroglobulin-Cleaving Antibodies in Autoimmune Thyroiditis

Thyroglobulin (Tg), the precursor of thyroid hormones, is an established target of Abs in autoimmune thyroid disease. Cleavage of ^{125}I-labeled Tg was studied by SDS-electrophoresis and autoradiography. Following treatment with Tg-specific autoantibodies isolated from a patient with Hashimoto's thyroiditis, disappearance of the 330-kDa Tg monomer and formation of a major 15-kDa product and minor 125-, 60-, and 25-kDa products were observed *(4)*. Control experiments showed the activity to belong to Tg-specific Abs. The K_m for Tg was 39 n*M*, indicating high-affinity Tg

recognition. The Tg Abs also cleaved Pro-Phe-Arg-MCA with low-affinity (K_m ~430-fold greater than for Tg). Pro-Phe-Arg-MCA hydrolysis was inhibited competitively and potently by Tg (K_i 20 n*M*), suggesting that the same catalytic site is responsible for cleavage of both substrates.

Inverse Relationship Between Antigen-Specific and Polyreactive Peptidase Antibodies in Autoimmune Disease

The author's lab has previously reported that patients with rheumatoid arthritis display decreased polyreactive proteolytic activity, determined by measuring the cleavage of a peptide-MCA substrate *(8)*. To compare the levels of Ag-specific and polyreactive Ab activities in the same patients, cleavage of the peptide-MCA substrate and Tg by IgG from healthy subjects, lupus patients, and autoimmune thyroiditis patients was determined *(38)*. Decreased peptide-MCA hydrolysis was evident in the SLE and autoimmune thyroiditis patients ($P < 0.002$). Both types of patients showed increased hydrolysis of Tg, compared to the control healthy subjects ($P < 0.001$). IgG samples purified from HIV-1-positive subjects or from mice hyperimmunized with an albumin–hapten conjugate did not cleave Tg, indicating that the development of the catalytic activity is not a nonspecific event accompanying V-region maturation.

The observations summarized above suggest increased expression of Ag-specific and efficient peptidase activities in lupus, autoimmune thyroiditis, and asthma. In contrast, comparatively nonspecific peptidase activities are decreased in autoimmune disease, compared to nondiseased controls. A possible explanation for this phenomenon is that genetic or cellular factors unique to autoimmune disease permit the increased recruitment of germline V-region genes encoding the unspecialized catalytic activity, followed by their specialization by autoantigen-driven clonal selection of B-cells. The hypothesis that autoimmune disease is associated with biased usage of different V genes is well established in the literature *(39)*. Other genes relevant to Ab expression may also contribute to catalytic activity levels in autoimmune disease. The MRL/*lpr* mouse is known to be a good catalytic Ab producer *(19)*. In this mouse strain, a mutation of the *Fas* apoptosis gene is believed to permit proliferation of T- and B-cells and expression of lupus-like disease *(40)*.

FUNCTIONAL SIGNIFICANCE OF AUTOANTIBODY CATALYSIS

The central focus of catalytic Ab researchers until now has been to validate and characterize the catalytic Abs, and only recently has attention been devoted to experimental study of the functions of the Abs.

The case of catalytic Abs to VIP is described here. Cleavage of any bond in VIP will lead to loss of its smooth-muscle relaxant and anti-inflam-

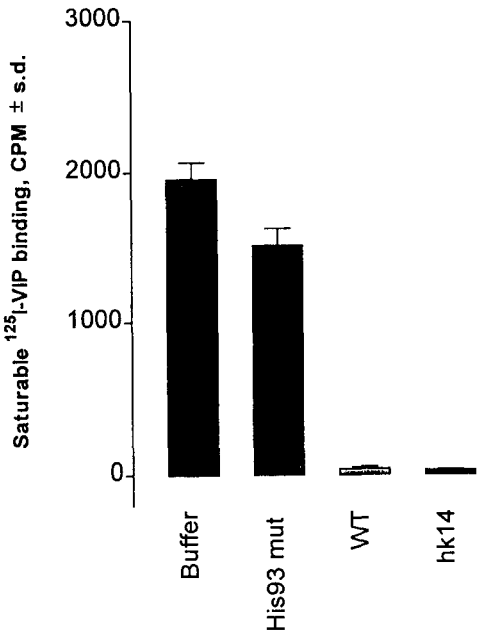

Fig. 3. Neutralization of VIP-receptor binding by peptidase L chains. WT, wild-type clone c23.5; His93 mut, catalytically deficient c23.5 mutant (His93:Arg); hk14, clone hk14 isolated from an asthma patient. ^{125}I-VIP (100 p*M*) was incubated (3 h) with assay buffer or the recombinant L chains (1 μ*M*). Receptor binding of the ^{125}I-VIP in 10-fold diluted reaction mixtures was measured by incubation with guinea pig lung membranes (33 μg protein), as in ref. *44*. Saturable binding represents the binding to receptors displaceable by excess unlabeled VIP.

matory effects, because the entire sequence of VIP is necessary for its biological activity *(41)*. Recombinant VIP-cleaving Abs with catalytic proficiency comparable to a non-Ab protease, trypsin, have been isolated *(21)*. Based on the magnitude of the catalytic activities, it is safe to predict that, when present, the catalytic autoantibodies may regulate the concentrations of the substrate polypeptide. In multiple myeloma, the Ab and L-chain products of the tumor cells accumulate in serum and urine to millimolar levels *(42)*. Some L chains isolated from myeloma patients express the ability to cleave VIP, an activity that might cause the depletion of the peptide in these patients.

High-affinity binding of neuronally released VIP by G-protein-coupled receptors expressed on airway cells stimulates cyclic AMP synthesis, and mediates, in part, the biological effects of VIP *(43)*. VIP-receptor binding by guinea pig lung membranes was measured as described in previous studies *(44)*, following incubation with the wild-type and His93:Arg mutant of the c23.5 L chain (Fig. 3). The mutant and wild-type L chains bind VIP with equivalent affinity, but the mutant is 100-fold less active cat-

alytically *(28)*. Near-complete inhibition of binding of VIP to the lung receptors by the wild-type protein was evident, whereas the mutant had minimal activity. It may be concluded that the catalytic function confers an enhanced VIP-neutralizing potency to the L chain.

Administration of a monoclonal antibody with VIP-cleaving activity to experimental animals increased the number of inflammatory cells and the thromboxane B2 levels in the airways, suggesting an inflammatory reaction *(45)*. These effects of the Ab are in line with the known actions of VIP. Release of VIP from nerve endings in the airway wall is believed to suppress inflammatory processes in the respiratory system, including release of inflammatory eicosanoid mediators *(46)*, interleukin production *(47)*, and the injurious effects of reactive oxygen radicals *(48)*. A deficiency of VIP may be responsible for increased inflammation in severe asthmatics *(49)*. Immunization of cats with VIP decreases the relaxation of airway smooth muscle in response to electrical stimulation *(50)*. VIPase Abs are also found in lupus patients, which is interesting because of the generalized tendency toward inflammatory processes and the frequent occurrence of respiratory disorders in lupus *(51)*.

A case could also be made that Ab catalysis is beneficial under certain circumstances. According to Grabar *(52)*, efficient autoantigen clearance will reduce the exposure of the immune system to autoantigens, and thus suppress autoimmune reactions. The possibilities of pathological vs beneficial effects are not mutually exclusive, because the Ab functions can vary, depending on their properties and biological location. Observations of increased occurrence of autoantigen-specific catalytic Abs in autoimmune disease, accompanied by decreased occurrence of the polyreactive catalytic Abs provide justification for the hypothesis that the former are harmful and the latter are beneficial. Tg, which fulfils its physiological function entirely within the thyroid, is found at very low levels in blood. Clearance of blood-borne Tg by catalytic Abs can arguably reduce further autoimmune reactions against this protein. On the other hand, if Tg-cleaving catalytic Abs permeate the thyroid or are made by thyroidal lymphocytes *(53)*, destruction of the protein will lead to depletion of the thyroid hormones T3 and T4, which are formed from Tg by an orderly iodination and peptidase-processing mechanism.

A possible role for catalytic Abs in Ag presentation can be conceived, although there is no experimental evidence for this at present. The hypothesis can be constructed that Ag digestion by Abs produces epitopes that bind major histocombatibility complex (MHC) Ags efficiently, thus permitting enhanced Ag presentation to T-cells and enhanced T-cell recruitment. Conversely, recruitment of autoantigen-specific T-cells will be reduced if the Abs generate epitopes incapable of binding MHC molecules.

CATALYST ENGINEERING

Notwithstanding the likely pathogenic nature of autoantibody catalysis, this phenomenon can potentially be put to beneficial uses. The purification of catalytic Abs suitable for immunotherapy of tumors and microbial infections has been a long-standing goal. The autoimmune repertoire available in the lupus MRL/*lpr* mouse strain is an appropriate starting point in the search for such Abs. Regardless of the reasons for the increased catalytic Ab synthesis, immunization with the desired tumor-specific or microbial Ag may yield catalytic Abs suitable for clinical applications.

A fruitful intersection of the fields of autoantibody catalysis and designer Ab catalysis can be conceived, in that immunization of autoimmune mice, with appropriate TSAs capable of recognizing the germline-encoded, serine protease site in Abs can be applied toward generation of efficient peptidase Abs (if human Abs are desired, the human Ab locus grafted in autoimmune mice can serve as the host).

The current generation of TSAs have been used to elicit catalytic Abs capable of catalyzing comparatively undemanding reactions like ester bond cleavage, but, because of various limitations in the structure of these TSAs, they do not elicit Abs that energetically cleave demanding reactions such as peptide bond hydrolysis (*see* refs. *54* and *55* for discussion). With the exception of one report showing the cleavage of a stable amide bond by an antiphosphinate Ab *(56)*, efforts to raise anti-TSA Abs capable of catalyzing complex reactions like peptidolysis and nucleolysis have not been successful. Note that a certain level of dogma has surrounded this issue: It has been argued that if anti-TSA Abs are deficient in such catalytic activities, ordinary antisubstrate Abs must be incapable of developing efficient enzymatic activities. This conceptual problem usually arises when the mechanistic differences between the naturally developed and engineered catalytic function are not appreciated clearly, i.e., the different immunological origins and the involvement of covalent vs noncovalent catalytic mechanisms in the two types of activities.

Catalysis can only occur if the reaction transition state is stabilized to a greater extent than is the substrate, but if a catalytic site is already encoded by the heritable Ab V genes (germline genes), it is straightforward to propose mechanisms by which the transition-state binding function is retained as the V genes are somatically mutated to acquire specificity for the ground state of an Ag over the process of clonal selection. Even improvements and *de novo* development in the catalytic function can be conceived if the catalytic function leads to the preferential proliferation of the maturing B-cells. The challenge for the engineer, then, is to develop reagents that can efficiently select for the prexisting catalytic Abs from the

immune repertoire, and can provoke the synthesis of Abs with improved catalytic activity relative to the germline configuration proteins.

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