CHAPTER 5

NATURE AND NURTURE OF CATALYTIC ANTIBODIES

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Abstract: Immunoglobulins (antibodies) frequently express constitutive functions. Two such functions are nucleophilic catalysis and the reversible binding to B-cell superantigens. Constitutive or "naturally-occurring" antibodies are produced spontaneously from germline genetic information. The antibody structural elements mediating the constitutive functions have originated over millions of years of phylogenic evolution, contrasting with antigen-driven, somatic sequence diversification of the complementarity determining regions (CDR) that underlies the better-known high affinity antigen binding function of antibodies. Often, the framework regions (FRs) play a dominant role in antibody constitutive functions. Catalytic antibody subsets with promiscuous, autoantigen-directed and microbe-directed specificities have been identified. Mucosal antibodies may be specialized to express high-level catalytic activity against microbes transmitted by the mucosal route, exemplified by constitutive production of IgA class antibodies in mucosal secretions that catalyze the cleavage of HIV gp120. Catalytic specificity can be gained by constitutive noncovalent superantigen binding at the FRs and by adaptive development of noncovalent classical antigen or superantigen binding, respectively, at the CDRs and FRs. Growing evidence suggests important functional roles for catalytic antibodies in homeostasis, autoimmune disease and protection against infection. Adaptive antibody responses to microbial superantigens are proscribed under physiological circumstances. Covalent electrophilic immunogen binding to constitutively expressed nucleophilic sites in B-cell receptors bypasses the restriction on adaptive antibody production, and simultaneous occupancy of the CDR binding site by a stimulatory antigenic epitope can also overcome the downregulatory effect of superantigen binding at the FRs. These concepts may be useful for developing novel vaccines that capitalize and improve on constitutive antibody functions for protection against microbes.

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INTRODUCTION

Structural stability of proteins is vital for maintaining higher-order life functions, and keeping proteins free of somatic mutations within the life-time of an individual is a hallmark of the disease-free state. The adaptive immune system is an exception. Immune molecules are specialized to mutate adaptively by somatic means, exemplified by the six complementarity determining regions (CDRs) of the immunoglobulin light and heavy chain variable domains (V_L and V_H domains). Within days to weeks, the CDRs acquire the ability to bind target antigens specifically with high affinity, a crucial property for defense against microbial infections.

The inherited antibody repertoire is usually conceived as a plastic starting point for adaptive development of specific, high affinity antigen binding sites formed by the CDRs. In humans, the inherited repertoire of germline genes that ultimately gives rise to the antibody V domains consists of about 50 light and heavy chain V_L and V_H region genes each, tens of diversity (D) genes, and tens of joining (J) genes. The lowest extant organisms in the phylogenetic tree of life expressing recognizable immunoglobulin molecules are the jawed fish (Fig. 1).[1] The ability to mutate antibodies had already

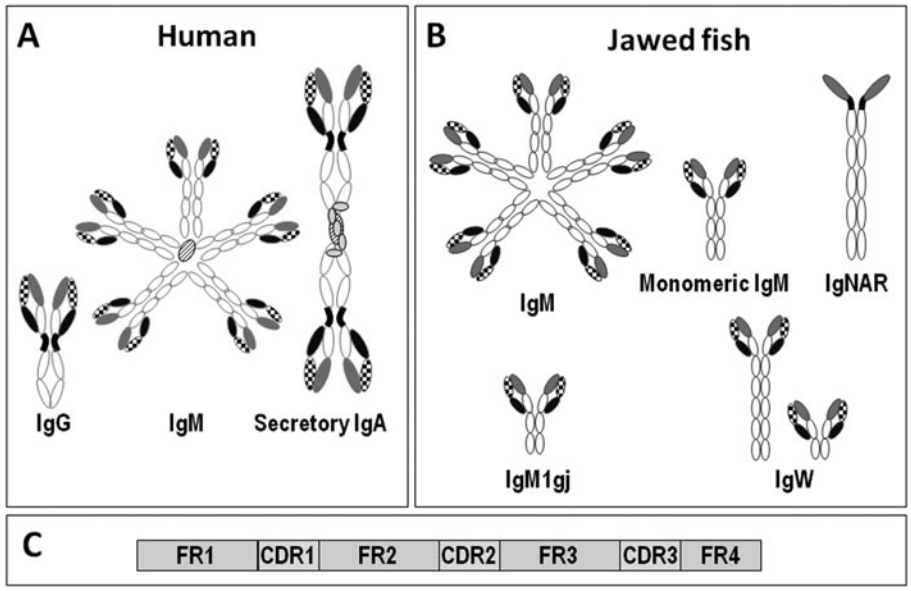

Figure 1. A) Domain organization of human IgM, IgG and secretory IgA. B) Domain organization of immunoglobulin isoforms in cartilaginous fish. C) Organization of complementarity determining regions (CDRs) and framework regions (FRs) of a typical variable (V) domain. Gray and white, respectively, heavy chain V and constant domains; Stippled and black, respectively, light chain V and constant domains. In fish (panel B), IgW refers to an isoform containing two forms of the heavy chain, a long seven domain form and a short 3 domain form. IgNAR (NAR, novel antigen receptor) is a heavy chain homodimer free of light chains. IgM1gj (gj, germline-joined) contains a V_H domain joined without somatic diversification at the V-D-J junctions. The monomeric IgM form is found in blood. Three L chain isotypes that cannot be classified as either κ- or λ-like have been identified in cartilaginous fish. Summarized from reference 1. In panel (C), binding of B-cell superantigens and traditional antigens is dominated, respectively, by the FRs and CDRs. A color version of this figure is available online at www.landesbioscience.com/curie.

appeared at this stage, albeit at a rudimentary level compared with higher organisms. No extant organism with immutable immunoglobulins has been identified. The missing link of a less sophisticated pre-form suggests an abrupt evolutionary advantage due to the development of somatically mutable immunoglobulins. This inspired John Marchalonis and coworkers to suggest the "big-bang" theory of adaptive immunity.[2] The concept of adaptive immunity mediated by the V domains is a central tenet of modern immunology, and it has been applied to great advantage in biotechnology, witnessed, for example, by the current > $15 billion/year market for therapy with monoclonal antibodies displaying specific, high affinity antigen binding activities acquired by adaptive means.

Interspersed between the V domain CDRs are the framework regions (FRs). Like the CDRs, the FRs undergo random sequence diversification by somatic hypermutation. However, classical antigens generally fail to select the FR mutations, as their primary contact amino acids are located in the CDRs. Thus, it is often stated that the FRs merely form a scaffold enabling the CDRs to fulfill their adaptive function in the somatic immune response. This view is no longer tenable given structural and functional evidence concerning subsets of antibodies with catalytic activity and reversible binding activity for microbial superantigens. These antibody subsets express constitutive V domain functions expressed without the requirement for antigen-driven somatic selection, often entailing a central participation of the FRs. There is growing realization of the beneficial and harmful effects of these functions in homeostasis, microbial infection and autoimmune disease (Table 1). FR and CDR encoded constitutive antibody functions have presumably improved over the course of phylogenic evolution under the influence of discrete selection pressures. CDR loop sequences, for instance, could undergo improvement if they enjoy superior conformational flexibility enabling adaptive development of binding to diverse epitopes in concert with random structural diversification. A more refined appreciation of the constitutive V domain functions may help explain long-standing immunological uncertainties and could offer innovative solutions for diseases that have remained intractable by conventional vaccine and immune therapy approaches, e.g., HIV infection, *Staphyloccus aureus* infection and Alzheimer disease.

CONSTITUTIVE OR "NATURALLY-OCCURRING" ANTIBODIES

There is no precise meaning of the terms "naturally-occurring" antibodies and "natural antibodies." The terms were coined to explain observations of antibody reactivities that can be differentiated functionally or structurally from those of antibodies induced by stimulation of the immune system with a known antigen. The "natural" and "induced" antibody subsets are both products of nature. Further, it is possible that certain "natural" antibodies" are actually "induced," e.g., their production may depend on autoantigen-driven adaptive selection of B cells. Therefore, the authors prefer to avoid these terms except when it is demonstrably clear that the antibodies are synthesized free of the influence of antigen. The first antibodies classified as "naturally-occurring" were polyreactive antibodies with the ability to bind two or more antigenic epitopes.[3,4] Polyreactive antibodies often recognize epitopes with no discernible structural resemblance. Thus, it is difficult to explain their binding pattern on the basis of cross-reactivity. Despite modest to low affinity, polyreactive antibodies are important for immunological defense because of their high concentrations in biological fluids. Indeed, large proportions of antibodies found in blood and mucosal secretions are thought to express polyreactive binding activity.

Table 1. Antibody subset expression and functions in various immune states. The classical antibody subset is composed of antibodies that develop high affinity antigen binding activity by traditional B-cell clonal selection. Constitutive antibodies included in the table are the catalytic, superantigen-binding and polyreactive antibody subsets.

Immune Phenotype	Constitutive Antibodies	Classical Antibodies
Homeostasis	• Polyreactive autoantigen binding • Promiscuous catalytic clearance of autoantigens • Catalytic mucosal defense against microbial superantigens	• No high affinity antibody synthesis
Autoimmune reactions	• Diminished catalytic clearance of small autoantigen peptides • Pathogenic, specific catalytic destruction of autoantigens • Beneficial, specific catalytic destruction of toxic autoantigens (e.g., amyloid) • Beneficial and harmful catalytic activation of zymogens	• Pathogenic autoantigen binding • Beneficial toxic autoantigen binding (e.g., amyloid)
Response to microbial infection	• Diminished catalytic clearance of small microbial peptides • Downregulated superantigen binding • Very slow adaptive amplification of superantigen catalytic cleavage • Adaptive catalytic response to CDR-binding antigens?	• Beneficial microbe binding
Response to vaccination	• Adaptive covalent vaccine-induced covalent and catalytic activity • Covalent vaccine-induced bypass of forbidden adaptive response to superantigens	• Beneficial microbe binding

Polyreactive antibodies are produced constitutively without stimulation by an exogenous antigen molecule. The clonal selection theory holds that antigen binding to the B-cell receptor (BCR; antibody complexed to signal transducing proteins expressed on the B-cell surface) drives B-cell clonal proliferation and adaptive selection of mutant BCRs expressing the greatest antigen binding affinity. The constitutively-expressed repertoire is generated by pairing of discrete V_L/V_H domains that are in turn produced from about 150 distinct V, D and J germline genes by V-(D)-J gene recombination, a step entailing extensive removal and addition of nucleotides defining the structure of CDR3 of each subunit. Structural diversity attributable to the V-(D)-J gene recombination and combinatorial pairing is constitutive or innate in the sense that it is produced randomly prior to arrival of the antigen on the scene. This innate, constitutive repertoire is enormous, estimated to be ~10^{13} B-cell clones expressing antibodies with unique structures.

The central point in resolving etymological debates about constitutive vs. somatically adapted antibody subsets concerns the role of antigen. Polyreactive antigen binding by constitutively produced antibodies depends at least in part on CDR3 of the V_H domain.[3] Therefore, V-D-J somatic diversification must contribute to acquisition of the polyreactive binding activity. Germ-free animals produce polyreactive antibodies, indicating that stimulation with exogenous antigens is not essential. A role for self-antigen in driving polyreactive antibody formation can be conceived, as these antibodies often bind self-antigens.[3,4] On the other hand, mitogen and cytokine stimulation alone is sufficient to drive differentiation of the constitutive B-cell clones into polyreactive antibody-producing plasma cells.[5] Also, the polyreactive BCR may be capable of autonomous positive signaling in a pre-BCR manner with no requirement of antigen signaling.[6]

BEYOND BINDING: CATALYTIC ANTIBODIES

The field of catalytic antibodies emerged from an interesting nurture vs. nature conundrum that lasted more than a decade. The idea that catalysts can be applied for efficient chemical transformation of biological and industrial molecules inspired chemical engineers to conduct immunizations with tetrahedral transition state analogs (TSAs) of ester substrates. The resultant antibodies were advertised as "nurtured" molecules with a tailored ability to catalyze ester hydrolysis attributable to noncovalent stabilization of the negatively charged transition state of the esterase reaction.[7] Later studies identified protease antibodies[8] and nuclease antibodies[9] that were generated by *bona fide* natural mechanisms with no reliance on immunization with artificial antigens. Mechanistic studies suggested that some antibodies raised by immunization with the TSAs actually use the same nucleophilic mechanism for chemical catalysis as naturally-occurring catalytic antibodies.[10] Moreover, it turned out that the immunogens thought to be chemically inert TSAs actually express electrophilic reactivity sufficient to bind covalently to the constitutive nucleophilic sites of antibodies.[11] This suggested that the TSAs stimulate adaptive improvement of the constitutive nucleophilic site. Clearly, nature played no small role in what was then thought to be the "nurtured" esterase function of the antibodies. A better example of nurtured catalysis may be the induction of a lactamase antibody by immunization with an anti-idiotypic antibody to a β lactamase enzyme.[12] In this molecular imprinting strategy, the anti-idiotypic antibody is assumed to express a surface complementary to the enzyme active site, thereby inducing an antibody with enzymatic activity.

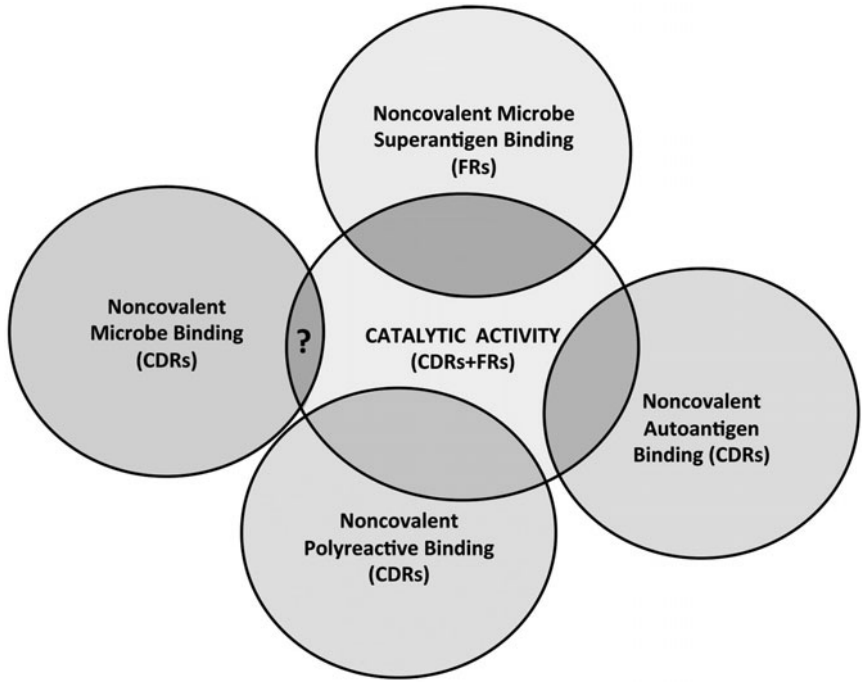

Figure 2. Antibody subsets with distinct functions and structural genesis. Note overlapping regions of the catalytic and other antibody subsets that impart to the catalytic antibodies their functional characteristics in homeostasis, autoimmune disease and microbial infections. The ? symbol denotes current lack of example antibodies that can effectively combine the catalytic function with noncovalent CDR-based recognition of microbial antigens. In contrast, numerous examples of autoantigen-specific catalytic antibodies are available. Assuming that autoantigen noncovalent binding occurs largely at the CDRs, it may be concluded that expression of the catalytic function in the CDR-driven autoimmune response is feasible. Both catalysis and ability to bind microbial superantigens noncovalently at the FRs are germline V gene functions expressed by constitutively-produced antibodies. Initial examples of combined expression of these functions are available. A color version of this figure is available online at www.landesbioscience.com/curie.

Kohler and Paul summarized the evidence for antibody interactions with ligands and substrates outside the classical antigen binding sites formed by the CDRs, including the interactions enabling chemical catalysis.[13] The immune genesis and functional roles of various catalytic antibody subsets is distinct from the classical antigen-binding antibodies (Fig. 2). As for classical antibodies, the initial step in catalysis by antibodies is noncovalent antigen binding (Fig. 3; noncovalent immune complex). Thereafter, a nucleophilic catalytic site in the spatial vicinity of the antigen binding site catalyzes the chemical conversion of the polypeptide antigen (Fig. 3; covalent immune complex 1 and 2).[14] The catalytic amino acids are located in part in the FRs and in part in the CDRs.[15-17] Catalysts turn over repeatedly to cleave multiple antigen molecules, and catalytic antibodies neutralize antigens more potently than conventional antibodies. Certain antibodies utilize the nucleophilic sites to form covalent immune complexes without proceeding to completion of the catalytic cycle. Such antibodies express enhanced antigen neutralizing activity due to their "infinite affinity" for the antigen. A review of catalytic antibodies and their relationship with other major antibody subsets follows.

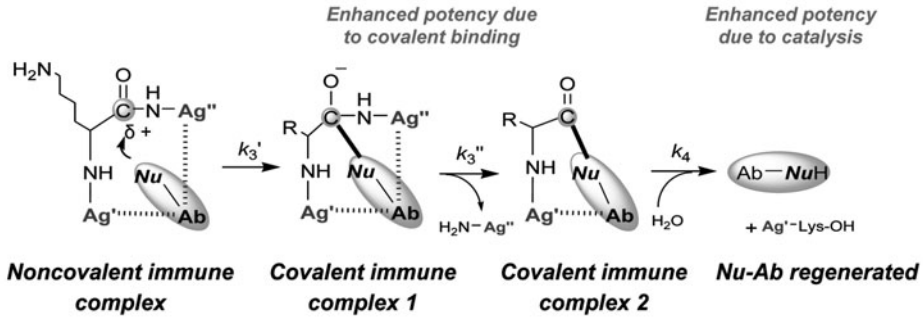

Figure 3. Mechanism of antigen-specific proteolysis by antibodies. Specificity is derived from noncovalent epitope–paratope binding. The antibody nucleophile attacks the weakly electrophilic peptide bond carbonyl. Covalent immune complex 1 is a resonant stable complex prior to expulsion of the C-terminal antigen fragment. Covalent immune complex 2 is an acyl-Ab complex. Ag' and Ag'' are components of the epitope recognized by the Ab. Ag'Lys-OH is the N-terminal antigen fragment and NH_2-Ag'' is the C-terminal antigen fragment. Covalent antigen binding alone is sufficient to enhance neutralizing potency compared with reversibly-binding antibodies, as the covalent reaction results in non-dissociable immune complexes. If catalysis occurs (that is, if active Ab is regenerated), this enhances potency further by reuse of a single catalyst molecule to permanently inactivate multiple target antigen molecules.

PROMISCUOUS CATALYTIC ANTIBODIES

Healthy humans and animals express catalytic antibodies that catalyze the hydrolysis of small amide and peptide substrates.[18-20] Other than requiring a positively charged Arg/Lys on the N-terminal side of the cleavage site, this catalytic antibody subset is promiscuous, displaying very low noncovalent binding affinity (Km values in the high micromolar range) and little or no dependence on the structure of the 'microantigen' substrate. Clearly, these antibodies fulfill their catalytic function without dependence on the classical adaptive development of noncovalent antigen recognition forces. The catalytic function itself is germline encoded. Each of the amino acids constituting the nucleophilic, catalytic triad of an antibody V_L domain are encoded by its germline V gene counterpart.[16] The adaptively-matured V_L contained certain somatic mutations remote from the nucleophilic triad. There was no loss of catalytic activity after reversion of the remote, somatically-derived amino acids to their germline counterparts.[16] Evidently, catalysis is a constitutive property acquired over phylogenic antibody evolution, not a de novo function developed by antigen-driven affinity maturation.

Studies on polyclonal antibodies isolated from blood provide initial insight to the fate of the catalytic function over the course of adaptive B-cell differentiation. IgMs, the first antibody class produced by B cells, expressed the promiscuous catalytic activity at high levels, whereas class-switched IgG antibodies were poorly catalytic.[19] Monomeric IgM also expressed superior catalysis compared with IgG, ruling out avidity effects as the cause of differing catalytic rates seen for the two antibody classes. IgMs and IgGs are distinguished by their differing constant domain architecture. The integrity of catalytic sites is dependent on precise, sub-Ångstrom-level spatial positioning of amino functional groups that is highly susceptible to changes in backbone movements, even movements caused by structural changes remote from the catalytic site. The feasibility of Ångstrom-level movements of V domain amino acid side chains upon attachment to the constant domains has been documented.[21] Therefore, a loss of catalytic site integrity due

to μ→γ constant domain replacement cannot be ruled out. Alternatively, as IgM→IgG class-switching occurs concomitantly with increasing accumulation of CDR mutations by the somatic hypermutation process, loss of the catalytic function activity may derive from remote CDR mutations.

Interestingly, blood-borne IgA antibodies express the promiscuous catalytic activity at levels even superior to IgM antibodies.[22] Improved activity of the class-switched, mature IgA antibodies is inconsistent with arguments of promiscuous catalysis as a vestigial property that could have been important at an early stage in antibody phylogeny but is functionally inconsequential in higher organisms. Patients with septic shock who die express low levels of promiscuous catalytic antibodies compared with patients who survive.[20] Patients with autoimmune disease also express lower levels of promiscuous catalytic antibodies than healthy individuals without disease.[18] A homeostatic function for promiscuous catalytic antibodies appears likely. Metabolic clearance of undesired proteins by antibodies with polyreactive antigen binding activity has been suggested, e.g., clearance of toxic microbial proteins and autoantigens that have outlived their utility.

Small peptides but not large proteins are cleaved rapidly by antibodies. IgA, IgM and IgG concentrations in blood are 2–10 mg/ml, compared with the nanogram/ml levels of classical protease enzymes. Promiscuous catalysis is readily detected at antibody concentrations magnitudes of orders lower than the physiological antibody concentrations. A strong case is available, thus, for metabolic clearance of small peptides by catalytic antibodies as a homeostatic function.

Consistent with observations that the free antibody light chain subunits express catalytic activities greater than intact antibodies,[23] robust peptide bond cleaving activities have been described for monoclonal light chains from multiple myeloma patients.[24,25] Some catalytic light chains induce cell death through an apoptotic pathway, an effect with potential significance for renal light chain accumulation and kidney failure in multiple myeloma patients.[26]

CATALYTIC AUTOANTIBODIES

The catalytic activity of antibodies was discovered as an autoantigen-specific function. Catalytic autoantibodies specific for vasoactive intestinal peptide (VIP),[8] DNA,[9] RNA,[27] thyroglobulin,[28] prothrombin,[29,30] Factor VIII,[31] Factor IX,[32] myelin basic protein,[33] and amyloid β peptide[34] have been reported. Catalytic antibodies may exert beneficial or deleterious effects depending on the biological context (see details below). Unlike the promiscuous catalysts in the preceding paragraph, these autoantibodies display catalytic specificity for individual autoantigens. Molecular interactions conferring specificity to the catalytic reaction can be understood from the split-site model,[35] in which noncovalent antigen binding and the subsequent catalytic cleavage step occur at two distinct but coordinated subsites (Fig. 4A,B). For the autoantigenic peptide VIP, mutations at certain antibody CDR residues resulted in loss of noncovalent binding activity without loss of catalytic rate constants.[15] Conversely, mutations at catalytic residues did not cause loss of binding affinity. Monoclonal antibodies with proteolytic activity cleave their specific substrate at multiple peptide bonds. Consistent with the split-site reaction model, the catalytic site can be oriented in register with alternate peptide bonds of the antigen in the noncovalently associated immune complex, resulting in cleavage at different bonds in the ensuing catalytic step of the reaction (Fig. 4C).

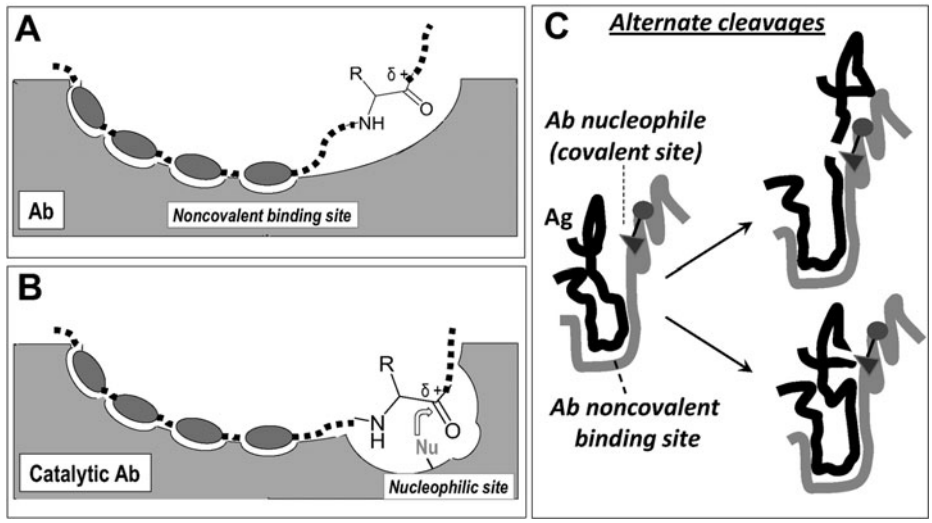

Figure 4. Split-site catalytic antibody model. A) Noncovalent epitope–paratope binding. Epitope component shown as a beaded structure. B) Noncovalent epitope–paratope binding coordinated with placement of a peptide bond of the antigen in register with the nucleophilic subsite (mostly localized in FR), initiating peptide bond hydrolysis. C) Two-step antigen recognition enabling cleavage at alternate peptide bonds. Intramolecular interactions impart nucleophilicity to an antibody residue (triangle activated by general base). Initial noncovalent binding rigidifies the peptide epitope but not the remote scissile peptide bond region. In the second step of the reaction, the nucleophile can be placed in register with alternate peptide bonds, resulting in cleavage at alternate sites by the antibody. Ab, antibody; Ag, antigen). A color version of this figure is available online at www.landesbioscience.com/curie.

Most proteolytic reactions produce functionally inactive products, e.g., the Factor VIII products obtained by antibody digestion are unable to mediate the cofactor function of intact FVIII in blood coagulation.[36] However, autoantibody-catalyzed cleavage of the precursor proteins prothrombin[29,30] and Factor IX[32] can generate the opposite result, that is, production of enzymatically active thrombin-like and Factor IXa-like products with the ability to promote coagulation. Catalytic autoantibodies were originally described as pathogenic mediators, analogous to the traditional conception of reversibly binding antigen-specific autoantibodies as mediators of Ehrlich's *horror autotoxicus* theory of autoimmunity. The Factor IX-activating catalytic autoantibodies found in acquired hemophilia patients, in contrast, are proposed to exert a beneficial pro-coagulant effect. Hemophilia in these patients is thought to be mediated at least in part by the anti-coagulant effect of FVIII binding autoantibodies. The procoagulant FIX-activating autoantibodies may be beneficial by counteracting the anti-coagulant effect of anti-FVIII autoantibodies.

The story of amyloid β peptide is also of interest. Accumulation of amyloid β aggregates in the brain is the hallmark of Alzheimer disease and amyloid β oligomer toxicity may underlie progressive neurodegeneration in this disease. Overproduction of amyloid β peptide with advancing age is devoid of any known physiological function. Catalytic autoantibodies that hydrolyze amyloid β peptide are proposed to be beneficial by virtue of their ability to impede formation of amyloid aggregates, dissolve the aggregates and block the toxic effects of the peptide oligomers.[34] Given that self-antigens can fulfill both

biologically essential and toxic functions, catalytic autoimmune reactions can be conceived to be functionally harmful when directed against biologically essential self-antigens and beneficial when directed against toxic self-antigens.

Unlike the non-covalent binding function, stimulation of the immune system by exogenous antigens generally fails to induce rapid improvement of catalysis by IgG antibodies. At the terminal step of the catalytic cycle, BCRs will release the antigen fragments produced by the proteolytic reaction. Adaptive improvement of catalysis, therefore, militates against the B-cell clonal selection theory, which holds that prolonged BCR occupancy by antigen is necessary for immune selection. Autoantibodies, on the other hand, can frequently express antigen-specific catalytic activity, and the frequent expression of catalysis by autoantibodies demands an explanation.

The answer may lie in electrophilic autoantigens that bind B cells and induce adaptive improvement of the germline encoded catalytic function. Engineered antigens containing artificial electrophiles are capable of inducing proteolytic antibodies by binding covalently to nucleophilic BCR sites.[14,19] This indicated that the nucleophilic reactivity underlying catalysis is selectable over the course of B-cell differentiation. Autoimmune diseases are often associated with increased post-translational generation of autoantigen adducts with lipid peroxidation metabolites and advanced glycation end products.[37] Such adducts contain reactive electrophiles capable of stimulating adaptive immune selection of catalytic antibody nucleophilicity. Yet another possibility is that under the abnormal B-cell regulatory pathways found in autoimmune disease, peptide bond cleavage by catalytic BCRs is itself a selectable event. Productive use of the energy liberated upon noncovalent antigen-BCR binding (binding energy) is central to initiation of signal transduction processes that eventually culminate in B-cell division and antibody synthesis. The process entails transduction of the binding energy into a remote conformational change that activates various stimulatory metabolic pathways. The cleavage reaction is a highly exothermic process. BCR signal transduction permitting productive use of the "catalytic energy" can be conceived, that is, the energy liberated from the catalytic reaction may cause a conformational change in the BCR that drives production of second messengers that in turn stimulate B-cell division. In view of accumulating evidence for autoantigen-specific catalytic autoantibodies, further study of non-traditional B-cell stimulatory mechanisms is warranted.

CATALYTIC ANTIBODIES IN DEFENSE AGAINST MICROBES

Do catalytic antibodies help the immune system fulfill its *raison d'être*, protection against microbes? As noted above, despite its promiscuous nature, the constitutive catalytic function of antibodies could potentially be useful in clearing small microbial peptides. Antibodies with DNase and RNase activity found in milk are proposed to hydrolyze viral and bacterial nucleic acids, although antibody access to internal microbial constituents was not shown.[38] Cleavage of protease-activated-receptor-2 by secretory IgA in milk induces a signal transducing pathway in intestinal epithelial cells that results in expression of anti-microbial β-defensins, which are known to help reduce neonatal infections.[39]

In addition, there is a strong connection between constitutive production of the subset of antibodies with catalytic activity and the subset that recognizes microbial B-cell superantigens by noncovalent means. Superantigens are defined as molecules recognized by antibodies with no requirement for adaptive B-cell differentiation or prior

exposure to the superantigen. Reversible antibody binding to the superantigenic sites of *Staphylococcus aureus* Protein A,[40] HIV gp120[41] and *Peptostreptococcus magnus*[42] is dominated by noncovalent interactions at antibody FRs, and certain CDR residues can also be involved. Like catalysis, superantigen binding is a constitutive antibody function derived from germline-encoded V domain elements. Discussion of the impact of reversibly binding and catalytic antibodies to a superantigenic epitope of HIV follows. The potential generality of catalytic antibody mediated defense against other microbes expressing B-cell superantigens remains to be investigated.

The immunodominant epitopes expressed by the HIV coat protein gp120 are highly mutable. However, the gp120 determinant that binds the primary HIV receptor on host cells, cell surface CD4, is essential for infection and is maintained in mostly constant form. The CD4 binding site (CD4BS) displays little structural variability in HIV-1 strains found across the world. This is one of the few immune vulnerabilities of HIV. Despite exposure of the CD4BS on the HIV surface, the immune system fails to mount a sufficiently protective antibody response to the CD4BS. The CD4BS core ($CD4BS^{core}$) is composed of residues 421–433. It overlaps the B-cell superantigen determinant of the protein.[43,44] Superantigens bind specifically to constitutively produced antibodies expressed as BCRs on the surface of B lymphocytes. Unlike the stimulatory binding of traditional antigens to the CDRs, superantigen binding at the FRs causes downregulation of B-cell differentiation, premature cell death and failure to mount an adaptive antibody response. We suggested that the constitutive ability of antibodies to bind superantigens was originally developed by Darwinian evolution processes over millions of years as a defense against primordial microbes.[45] The superantigenic $CD4BS^{core}$ epitope may be HIV's answer. Development of a CD4BS with superantigenic character leaves HIV open to constitutive immunity, but it also minimizes virus neutralization by downregulating the adaptive antibody response.

A subset of the antibodies to the superantigenic $CD4BS^{core}$ proceed to catalyze the breakdown of peptide bonds, destroying gp120 permanently.[46] The catalytic sites are present in antibodies produced without requirement for prior HIV infection.[46,47] Sexual transmission of HIV generally occurs through the rectal and vaginal mucosal surfaces. Only a minority of sexual intercourse events with an infected individual causes virus transmission. Remarkably, secretory IgA class antibodies found at mucosal surfaces of non-infected humans rapidly catalyze the cleavage of gp120 and neutralize HIV in tissue culture.[47] Blood-borne IgA and IgM antibodies display lower catalytic proficiencies. A single catalytic antibody molecule is reused to cleave thousands of gp120 molecules. The neutralization potency of catalytic antibodies, therefore, is superior to traditional antibodies that bind the antigen reversibly on a 1:1 basis. It may be hypothesized that the catalytic secretory IgAs represent a constitutive defense against mucosal HIV transmission.

The $CD4BS^{core}$ appears to be the proverbial Achilles heel of the virus. Potent HIV neutralizing antibodies have been isolated from non-infected patients with lupus,[48] an autoimmune disease that is associated with resistance to HIV infection[49] and increased catalytic antibody production.[50] Catalytic antibody light chains to the $CD4BS^{core}$ have been reported.[51] It is not clear whether an autonomous BCR signaling event or a discrete antigen drives amplification of the antibodies. No structural homology is evident between the $CD4BS^{core}$ and human protein sequences available in the databanks. However, we found partial $CD4BS^{core}$ sequence identity with certain human endogenous retroviral sequences (HERVs).[45] HERVs are evolutionary remnants of retroviral integration into the

host genome. About 2–10% of the human genome is composed of HERVs, and HERV expression is amplified in lupus patients.[52]

An explicit example of adaptive production of antibodies to the superantigenic CD4BS^{core} is provided by patients who survived HIV infection over a prolonged period of about two decades.[53] IgA class antibodies specific for the CD4BS^{core} identified in the blood of the survivors neutralized heterologous viral strains potently. The IgAs contained an antibody subset that binds gp120 non-covalently and another antibody subset that proceeds to hydrolyze gp120. It appears that adaptive production of the antibodies to the CD4BS^{core} is proscribed, but in the rare circumstances that adaptive anti-CD4BS^{core} antibodies are generated, they neutralize HIV strains from across the world with exceptional potency. The immune response to microbial infection is a stochastic process relying on occurrence of immunologically favored, high probability B lymphocyte differentiation events. Low probability events are manifested with passage of time. Understanding how B cells from HIV survivors bypass physiological restriction on adaptive production of antibodies to the superantigenic CD4BS^{core} could furnish insight to design a vaccine that induces similar antibodies. The potential bypass mechanisms are: (a) The B cells may slowly produce antibodies that bind the CD4BS via their CDRs with no utilization of the pre-existing CD4BS binding site programmed genetically into the FRs; and (b) Cellular downregulation due to CD4BS binding to the antibody FR site may be effectively counteracted by a favorable differentiation signal generated upon simultaneous engagement of another epitope on the same gp120 molecule by the CDRs.

In addition to gp120, two other HIV proteins essential for virus infection are cleaved by catalytic antibodies from HIV infected patients, reverse transcriptase and integrase.[54,55] Sufficient details of patient clinical history and antibody specificity have not been provided, making it difficult to assess whether the catalytic antibodies are produced via the classical adaptive response pathway. Also, the authors reported enrichment of the catalytic antibody subset in the Protein A binding fraction, raising questions whether Protein A binding to discrete V domain FRs responsible for superantigen epitope recognition was a contributory factor in identifying the catalysts. There is no evidence for the expression of superantigenic epitopes by HIV integrase or reverse transcriptase, but this is not an inconceivable feature of these proteins. Note that a rapid adaptive catalytic antibody response to microbial antigens akin to the classical adaptive binding response is feasible only if the catalytic event itself is immunologically selectable via productive use of the catalytic energy for driving B-cell maturation.

CATALYTIC ANTIBODIES AS SECOND GENERATION THERAPEUTICS

Reversibly binding monoclonal antibodies and polyclonal IgG from healthy humans have emerged as a paradigm for therapy of cancer, neurological disease and autoimmune disease. Yet cost and limited efficacy are substantial concerns. Catalytic antibodies hold potential for improved efficacy, and in some instances, lesser side effects. According to Thomas Kuhn, for a new paradigm candidate to be accepted by a scientific community, "First, the new candidate must seem to resolve some outstanding and generally recognized problem that can be met in no other way. Second, the new paradigm must promise to preserve a relatively large part of the concrete problem solving activity that has accrued to science through its predecessors." With the exceptions noted

below, the current therapeutic antibody paradigm derives from the notion of antibodies as "magic bullets" that target defined antigens with minimal collateral damage to other antigens. A high level specificity for individual antigens, therefore, is an important property for minimizing catalytic antibody side effects. Maximizing efficacy will depend on the rapidity of catalysis. Any future paradigm of catalytic antibodies as second generation therapeutics will depend on identifying antibody preparations with sufficient specificity and turnover rate.

Catalytic IVIG?

Intravenously administered formulations of pooled human IgG (IVIG) are largely composed of constitutively produced antibodies. IVIG provides incremental benefit in patients with certain autoimmune diseases and immunodeficiency states. In addition, the use of IVIG and its IgM-containing counterpart IVIGM for treatment and prevention of bacterial infection has been debated. The manufacturing procedure includes use of the Cohn-Oncley cold ethanol fractionation method developed in the 1940s. While V domain binding functions are usually maintained, IVIG is essentially devoid of catalytic activity,[22] presumably because the catalytic sites are disrupted by stringent solvent treatments. A commercial IVIGM preparation also displayed minimal catalytic activity. Moreover, IVIG is generally formulated using only IgG class antibodies, which express catalytic activities orders of magnitude lower than native IgM and IgA antibodies. As the promiscuous catalytic function of endogenous antibodies is likely to fulfill a homeostatic role, it is reasonable to examine the potential benefits of catalytic IVIG formulations composed of IgM/IgA class antibodies purified under non-denaturing conditions.

Alzheimer Immunotherapy

No therapy for a sustained improvement of cognition is available for Alzheimer's disease. In amyloid β overexpressing transgenic mice, small amounts of intravenously infused monoclonal antibodies that bind amyloid β peptide enter the brain, clear brain deposits of the peptide and improve cognition.[56] However, human trials have raised concern about limited therapeutic efficacy and side effects. A monoclonal IgG to the amyloid β peptide modestly reduced the risk of cognitive decline in patients negative for the ApoE4 allele, a genetic trait associated with delayed onset of Alzheimer disease.[57] Microglia, the brain's resident macrophages, fulfill the beneficial function of clearing antibody-amyloid β immune complexes by uptake through their Fc receptors. However, microglial activation by this pathway holds the risk of inflammatory mediator release, potentially exacerbating the already inflamed state of the Alzheimer brain. Immune complex deposition in the vascular walls can cause cerebral microbleeds. Provided that the antibodies cleave amyloid β peptide with sufficient specificity, catalytic antibodies hold many of the advantages and none of the disadvantages of reversibly binding antibodies. Because catalysts do not form long-lived immune complexes, the risks of vascular immune complex deposition and microglial release of inflammatory mediators occurring as a result of Fc-receptor mediated immune complex uptake is minimized. High turnover catalytic V_L domains to amyloid β with serine protease character have been isolated using phage display library methods[21] and a single chain catalytic Fv is also available[58] for further development.

Infectious Disease

Uda and coworkers have developed a strategy to isolate high turnover catalytic subunits from monoclonal antibodies. They apply molecular modeling to identify potential catalytic triads in the antibodies raised to ordinary polypeptide antigens, prepare the individual heavy and light chains, and screen for catalytic cleavage of the polypeptide antigen. The strategy was successful in preparing catalytic light chains capable of rapid cleavage of *Helicobacter pylori* urease. This bacterium is the etiologic agent in several gastroduodenal diseases. Oral administration of the light chain reduced the number of *H. pylori* in the stomachs of mice.[59]

HIV immunotherapy is plagued by the problems of poor potency and emergence of antibody-resistant viral mutants. Can catalytic antibodies be used for passive HIV immunotherapy? The answer depends on the epitope specificity and neutralizing potency of the catalysts. Targeting the $CD4BS^{core}$ minimizes the opportunity for developing antibody-resistant strains, as mutations in the $CD4BS^{core}$ are predicted to result in loss of viral infectivity. Indeed, anti-$CD4BS^{core}$ antibodies from long-term survivors of HIV infection neutralized the autologous HIV strain potently, arguing against emergence of resistant strains despite the selective pressure imposed by the antibodies over prolonged durations. Catalytic monoclonal antibodies to the $CD4BS^{core}$ raised by immunization with an electrophilic peptide mimetic neutralize diverse HIV strains in tissue culture, supporting their potential therapeutic utility.[60,61]

HIV infected individuals produce catalytic antibodies to the viral integrase and reverse transcriptase enzymes. Intracellular expression of catalytic antibodies to these proteins holds potential for early blockade of viral propagation via interference with copying viral RNA into proviral DNA and DNA integration into the host genome. Gene therapy protocols for intracellular antibody expression[62] can be conceived for persistent delivery of catalytic anti-HIV antibodies. Reactivation of HIV infection can occur due to integration of the viral genome into host DNA. Drugs that deplete proviral DNA reservoirs are under investigation to address the problem of HIV latency.[63] Catalytic antibodies combined with a proviral DNA-depleting drug may be suitable for consideration as an alternative therapy for the infection.

CATALYTIC AND COVALENT VACCINATION

In the case of HIV, a clear path to a vaccine that induces broadly neutralizing antibodies can be foreseen if the following milestones can be reached: (a) Reproduction of the correct $CD4BS^{core}$ conformation in the vaccine candidate, and (b) Rapid adaptive production of neutralizing anti-$CD4BS^{core}$ antibodies upon administration of the vaccine candidate. Preclinical studies of the 'covalent vaccination' strategy are promising.[60,61] Central points are:

- The covalent binding of B cells by an electrophilic polypeptide containing the $CD4BS^{core}$, the vaccine candidate, induces production of broadly neutralizing antibodies. The polypeptide is activated chemically by linking lysine side chains to the strongly electrophilic phosphonate diester group. Naturally-occurring nucleophilic sites are found ubiquitously in BCRs.[19,64] Noncovalent $CD4BS^{core}$ epitope binding to BCRs positions the electrophilic group within covalent binding

distance of nucleophilic groups. Like the catalytic reaction discussed above, covalent bonding between the electrophile and nucleophile liberates a very large amount of energy. Alone, noncovalent CD4BS^{core} binding to BCRs does not stimulate antibody synthesis. As administration of covalently reactive CD4BS^{core} containing antigens to experimental animals induced neutralizing anti-CD4BS^{core} antibodies, it appears that the binding energy released from the covalent antigen-BCR reaction is used productively to induce a remote BCR conformational change that stimulates signal transduction processes permitting differentiation of B cells into plasma cells producing class-switched neutralizing antibodies.

- A unique feature of the strategy is recruitment and clonal expansion of the small subset of B cells capable of producing antibodies with constitutive, pre-existing specificity directed to the CD4BS^{core}. The induced antibodies neutralized diverse HIV strains, a long-sought objective in HIV vaccine research (see example of a neutralizing antibody in Fig. 5A). The CD4BS^{core} binds at a site located mainly in the FRs. Neutralizing antibody production occurs without dependence on typical adaptive mutational processes occurring in the CDRs. However, adaptive improvement of the antibodies due to FR mutations is feasible, indicated by evidence from immunization of animals with electrophilic gp120 and an electrophilic CD4BS^{core} peptide mimetic.[60,61] Robust neutralization of diverse HIV strains by the antibodies in tissue culture was evident. The antibodies displayed specific recognition of the CD4BS^{core}, confirming mimicry of the native CD4BS^{core} by the vaccine candidates.

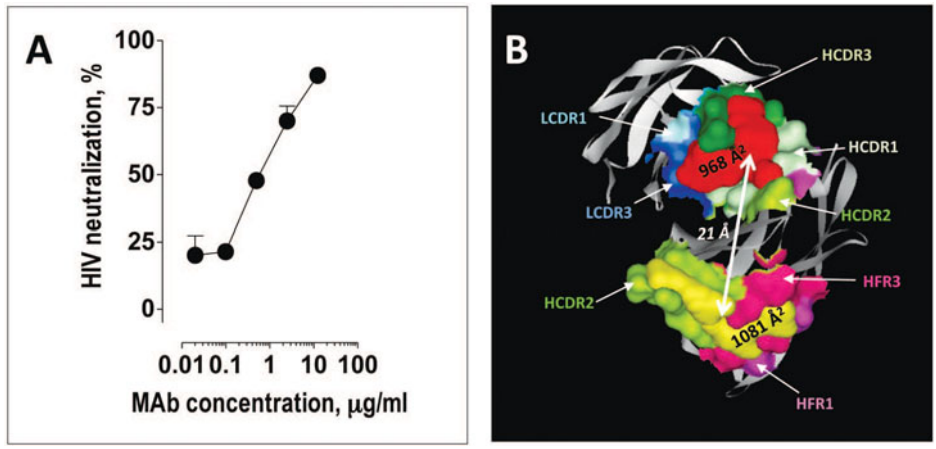

Figure 5. Binary epitope reactivity of neutralizing antibodies raised by immunization with electrophilic gp120. A) Representative HIV neutralization data for monoclonal antibody 3A5. Clade C, CCR5-dependent HIV, strain ZA009. Host cells, peripheral blood mononuclear cells. B) Fab YZ23 structure solved by crystallography (2.5Å resolution, PDB 3CLE) showing the classical antigen binding cavity formed by the heavy and light chain CDRs (HCDR3, HCDR1, a small HCDR2 segment and LCDR3) filled with a red object and a second cavity dominated by heavy chain FR1 and FR3 (HFR1, FFR3) filled with a yellow object. A segment of heavy chain CDR2 (HCDR2) distant from the classical antigen binding cavity helps form the second cavity. The CDR-dominated and FR-dominated cavity bind, respectively, gp120 residues 301–311 and the CD4BScore. Double headed arrow indicates inter-cavity centroid to centroid distance. Cavity surface areas are indicated within red and yellow objects. Neutralization data and the crystal structure are from reference 60.

- Immunization with full-length electrophilic gp120 revealed another novel mechanism for overcoming the physiological hurdle in producing anti-CD4BS^{core} antibodies.[60] Neutralizing antibodies to electrophilic gp120 displayed binary epitope reactivity, that is, the ability to bind the CD4BS^{core} at the antibody FRs and a second spatially distant epitope composed of gp120 residues 301–311 at the antigen binding cavity formed by the CDRs (Fig. 5B). The binary reactivity suggests that simultaneous stimulatory binding of the second immunogenic epitope at the CDRs compensates for the downregulatory CD4BS^{core} binding at the framework regions.
- In addition to inducing reversibly-binding antibodies, covalent binding of the electrophilic vaccine candidate selects BCRs with the greatest nucleophilic reactivity.[14,65] In turn, the improved nucleophilic reactivity enhances antibody inactivation of HIV as follows. First, specific pairing of the antibody nucleophile with the weakly electrophilic carbonyls of gp120 forms stable immune complexes with covalent character (Fig. 3). Binary epitope-reactive antibodies were induced by immunization with the electrophilic analog of full-length gp120 and similar antibodies with single epitope reactivity were induced by immunization with an electrophilic analog, a synthetic gp120 peptide corresponding to an immunodominant epitope located in the third variable domain of gp120.[14,65] As the covalent bond is very strong, the antibody-HIV complexes did not dissociate, increasing the HIV neutralization potency. Second, the subset of antibodies, containing combining sites that support water attack on the covalent gp120-antibody complex, is capable of catalyzing gp120 cleavage. A subset of catalytic antibodies capable of rapidly catalyzing the cleavage of gp120 was obtained by immunization with the electrophilic CD4BS^{core} peptide.[61]

Enzyme active sites have evolved to undergo precise active site conformational readjustments that fulfill the changing requirements of individual steps in the catalytic reaction cycle, that is, initial noncovalent binding, nucleophilic attack and transition state binding, water attack on the covalent acyl-antibody complex and product release (Fig. 3). Note that inducing efficient antibody catalysis is more feasible in case of superantigen epitope targeting,-as catalytic antibodies to the superantigens are produced constitutively. For such targets, the electrophilic antigen analog must merely amplify the pre-existing, constitutive subset of catalytic antibody producing B cells. For non-superantigenic targets, de novo induction of the catalytic function is required, a more onerous hurdle. Monoclonal anti-gp120 IgGs with slow catalytic activity have been identified following immunization with an electrophilic analog of full-length gp120[14] and a gp120 pentapeptide without evident superantigenic character.[66] Successful vaccination requires induction of robust polyclonal antibody responses in biological fluids. The monoclonal antibodies with low-level catalytic activity do not justify projections of vaccine-relevant antibody catalysis. Indeed, monoclonal antibodies capable of slow, antigen-specific proteolytic activity are induced even by routine immunization with polypeptides devoid of exogenously-introduced electrophiles.[67,68]

Despite caveats concerning induction of efficient de novo catalysis, the electrophilic antigen analogs offer indisputable value by virtue of their ability to induce adaptively strengthened antibody nucleophilic activity resulting in covalent recognition of the target antigen.[65,69] This applies regardless of the superantigenic character of the target. Side-by-side immunizations using an electrophilic antigen analog and the control antigen

devoid of exogenous electrophiles revealed the superiority of the former immunogen, evident from enhanced antigen neutralization by polyclonal antibodies attributable to covalent immune complexation.[65]

CONCLUSION

The field of antibody catalysis has a secure place alongside other constitutive antibody functions in helping develop novel concepts that offer insight to immune homeostasis, autoimmune reactions and protection against infection. The field is also increasingly closer to realizing its utilitarian potential for developing novel therapies and vaccine strategies directed at intractable diseases.

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CONFLICT STATEMENT

Sudhir Paul, Stephanie Planque and Yasuhiro Nishiyama have a financial interest in patents covering the covalent immunization and catalytic antibody areas. Sudhir Paul and Richard Massey have a financial interest in Covalent Bioscience Inc., and Sudhir Paul is a scientific advisor for the company. Carl V. Hanson declares no competing financial interest.

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