Beneficial Catalytic Immunity to A β Peptide

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Abstract

We review attempts to treat Alzheimer disease with antibodies that bind amyloid β peptide (A β) and the feasibility of developing catalytic antibodies for this purpose. Naturally occurring immunoglobulin M (IgM) class antibodies that hydrolyze A β and inhibit A β aggregation were identified. The production of these antibodies increases as a function of age, ostensibly reflecting an attempt by the immune system to protect against the deleterious effect of A β accumulation in old age. A search for catalytic antibodies in a library of human immunoglobulins variable (IgV) domains yielded catalysts that hydrolyzed A β specifically at exceptionally rapid rates. The catalytic IgVs contained the light-chain variable domains within scaffolds that are structurally reminiscent of phylogenetically ancient antibodies. Inclusion of the heavy-chain variable domain in the IgV constructs resulted in reduced catalysis. We present our view that catalytic antibodies are likely to emerge as more efficacious and safer immunotherapy reagents compared to traditional A β -binding antibodies.

Introduction

ALZHEIMER DISEASE (AD) IS THE commonest age-induced dementia, with an estimated worldwide prevalence of approximately 26 million humans. Acetylcholinesterase inhibitors provide symptomatic relief for AD. However, no available AD therapy rectifies the dysfunctional processes underlying the disease. Accumulation of amyloid β (A β) peptide aggregates is thought to play a central role in the pathogenesis of AD.¹ Even physiological aging may be associated with increased A β .² The A β aggregates are composed of 39- to 43-residue peptides generated by proteolytic processing of the amyloid precursor protein (APP) by β - and γ -secretases.³ The predominant product of this processing pathway is the 40-amino-acid peptide corresponding to APP residues 597–636 (A β 40), with the 42-amino-acid peptide corresponding to residues 597–638 (A β 42) being the next most abundant product. Both peptides form toxic oligomeric aggregates and fibrillar aggregates found in amyloid plaques characteristic of the AD brain.³ A β 42 tends to aggregate more rapidly and is the majority species in amyloid plaques.³ A β 40 is the major species found in peripheral blood.⁴ At very low concentrations, A β can exert trophic effects on the cells.^{5,6} However, A β overproduction occurs due to dysregulated neuronal metabolism, and there is no known physiological advantage of A β accumulation in the aged brain. Death of neurons cultured with synthetic A β aggregates has been reported.⁷ Soluble A β oligomers can induce neurodegenerative effects by several pathways, including altered expression of memory-related receptors⁸ and induction of aberrant neuro-

nal responses to electrical stimulation.⁹ We review here the status of AD treatment with antibodies that bind A β and the potential of catalytic antibodies for inducing an improved therapeutic effect.

Amyloid-Binding Antibodies for Immunotherapy of AD

Removal of A β from the brain has been advanced as a potential treatment of AD. Studies in transgenic mice expressing mutant human amyloid precursor protein genes (APP-Tg mice) suggest that A β -binding antibodies clear brain A β deposits and correct the behavioral deficits evident in this animal model. The favorable effects were observed following peripheral administration of A β -binding monoclonal antibodies^{10,11} (passive immunotherapy) and after active immunization with A β itself (active immunotherapy), which induces the synthesis of A β -binding antibodies.^{12–14} The effects were evident when the antibodies were administered both prior to¹¹ and after¹⁰ the appearance of A β plaques in the murine brain. These findings lead to clinical trials of active A β immunotherapy as a treatment for AD. Two important points emerged from the human trials.¹⁵ First, only about 20% of the recipients developed A β -binding antibodies, reflecting the limited immunogenicity of the A β vaccine formulation. Second, the trials were suspended because $\approx$ 5% of the immunized patients developed sterile meningoencephalitis, suggesting an inflammatory reaction. Patients who produced A β -binding antibodies displayed reduced decline of certain cognitive functions,¹⁶ but the therapeutic benefit has been subject to debate.¹⁷

Antibodies with A β -binding activity can cause undesirable side effects,¹⁸ and there is also the potential of harmful cell-mediated immunity after immunization with A β . The latter concern is eliminated if preformed A β -binding antibodies are employed for passive immunotherapy. A Phase II trial of Bapineuzumab, a humanized reversibly binding monoclonal A β -binding immunoglobulin G (IgG), administered intravenously to mild-to-moderate AD patients has been conducted.¹⁹ There was no indication of unacceptable inflammatory reactions, but a dose-limiting incidence of vasogenic edema was evident. This effect may be due to microbleeds caused by deposition of immune complexes in cerebral blood vessels (Fig. 1). AD patients homozygous for the apolipoprotein E4 allele are predisposed to increased A β accumulation and early development of AD.²⁰ Administration of the A β -binding antibody to patients who were not homozygous for the apolipoprotein E4 allele resulted in significantly reduced cognitive decline and a lesser tendency toward vasogenic edema. The favorable effect on cognition did not reach statistical significance in the intent-to-treat population. From these results, there is cautious support for passive AD immunotherapy using A β -binding antibodies, but the safety and efficacy of the procedure leave room for improvement.

Eli Lilly has advanced its own humanized monoclonal IgG into Phase III trials. This IgG binds an epitope located in the middle region of A β , whereas Bapineuzumab binds the A β

amino terminus.²¹ Detailed information about the beneficial and side effects of the Lilly monoclonal IgG has not been released, but the company has indicated that antibody administration induces an increase of A β levels in cerebrospinal fluid and peripheral blood.²² To the extent that the observed increase of peripheral A β is due to release from the brain peptide stores, the antibody may exert a favorable effect.

Interestingly, healthy humans and AD patients produce A β -binding autoantibodies spontaneously.²³ Pooled human IgG marketed as intravenously administered immunoglobulins (IVIG) formulations contains small amounts of A β -binding autoantibodies. Early-stage clinical trials entailing intravenous administration of very large IVIG doses to AD patients (e.g., 1.2 grams/kg over 3 days) were encouraging, but no definitive evidence for efficacy is available yet.²⁴ No side effects have been reported.

A β -binding IgGs are proposed to reduce A β deposition in the brain by the following mechanisms (Fig. 1)^{25,26}: (1) Small amounts of peripherally administered IgGs may cross the blood-brain barrier (BBB; $\approx 0.1\%$ of injected IgG dose) and bind A β in the brain. Microglial cells then ingest the immune complexes via an Fc γ -receptor mediated process that results in A β clearance; (2) the IgGs can also bind the neonatal Fc receptor (FcRn) located on the abluminal (brain) side of the endothelial cells constituting the BBB, thereby facilitating A β efflux into the periphery; (3) A β binding to IgG may constrain

FIG. 1. Passive immunotherapy of Alzheimer disease (AD) with amyloid β peptide (A β)-binding immunoglobulin G (IgG). Reversibly binding IgG injected into peripheral blood can enter the brain in small amounts and help clear A β by mechanisms listed in the text. Following binding of IgG to A β monomers (a) or aggregates (b), the immune complexes are ingested by an Fc γ receptor-dependent uptake process into microglia (c). The IgG bound by FcRn receptors at the blood-brain barrier may help clear A β from the brain and efflux to the periphery via transcytosis (d). Microglial ingestion of the immune complexes is accompanied by release of inflammatory mediators, which could exacerbate the already inflamed state of the AD brain. In mouse AD models, clearance of amyloid plaques from the brain parenchyma induced by A β -binding IgGs can be accompanied by A β deposition in the blood vessels and microhemorrhages. FcRn, Neonatal Fc receptor; LRP-1, low-density lipoprotein receptor-related protein 1; RAGE, receptor for advanced glycation end products.

the peptide into a nonaggregable conformation; and (4) according to the "peripheral sink" hypothesis,²¹ A β is cleared from the brain without IgG entry into the brain. In this hypothesis, A β binding by antibodies in peripheral blood perturbs the equilibrium between the peptide pools in the brain and periphery, thereby inducing A β release from the brain. In principle, these mechanisms are not mutually exclusive and may be triggered by the same antibody.

Catalytic Antibody Overview

The feasibility of developing catalytic antibodies for immunotherapy is based on the following immunological and biochemical findings:

1. Antibodies express catalytic activities akin to enzymes as a natural property.^{27,28} The innate antibody repertoire developed over millions of years of immune evolution is composed of antigen recognition sites derived from about 150 rearranged germ-line genes encoding the variable (V) domains of the light- and heavy-chain subunits (V, D, and J genes) that are expressed as fusion products containing the μ , δ , γ , α , and ϵ constant domains. Antibody V domains in their germ-line gene configuration can express catalytic activity.^{29,30}
2. Antigen-specific catalysis occurs by a split-site mechanism entailing initial noncovalent binding followed by proteolysis occurring at a distinct but spatially neighboring nucleophilic site of the antibodies.^{29,31} Antigens are recognized by antibody combining sites composed of the paired V domains of the light and heavy chains (V_L and V_H domains). Most commonly, the catalytic sites are located in the V_L domains.^{27,28} Certain polypeptides recognized specifically by antibodies present in the innate immune repertoire are designated B cell superantigens, e.g., the human immunodeficiency virus (HIV) coat protein gp120.³² Such superantigens are subject to proteolytic degradation by antibodies.^{33,34}
3. Naturally occurring antibodies usually employ proteolytic mechanisms akin to the serine protease family of enzymes, an example of convergent molecular evolution. The intramolecular hydrogen-bonded network at the catalytic site imparts nucleophilic reactivity to the serine (Ser) residue, allowing covalent attack on the peptide bond. A Ser-histidine (His)-aspartic acid (Asp) catalytic triad has been identified by mutagenesis in proteolytic antibody IgG.^{29,30} Hapten electrophilic phosphonates originally synthesized as probes for enzymatic nucleophiles inhibit catalysis irreversibly by binding the nucleophilic site.³⁵ Development of structurally divergent antibody nucleophilic sites is conceivable, consistent with the ability of various other amino acids to serve as nucleophiles and the activating groups.³⁶ An atypical Ser-Arg-Glu catalytic triad has been identified in crystals of a proteolytic antibody.³⁷ Atypical metal-assisted proteolytic antibodies have also been described.³⁸
4. Catalytic antibodies can be induced by immunization with ordinary antigens or antigen intermediates formed along the pathway of the catalytic reaction.³⁹⁻⁴² However, there are important limitations in the adaptive improvement of antigen-specific proteolytic antibodies. Antigen binding to the B cell receptor (BCR; surface

immunoglobulins associated with signal transducing proteins) drives B cell division. BCR-catalyzed antigen hydrolysis will result in release of the antigen fragments, depriving the cells of the proliferative signal. In the most immune responses, therefore, B cells that express catalytic BCRs are subject to deletion.

5. The first example of antibody proteolysis was the finding of autoantibody catalyzed hydrolysis of the neuropeptide vasoactive intestinal peptide (VIP).⁴³ Interestingly, most other examples of naturally occurring catalytic antibodies are also found as autoantibody activities.^{27,28} It may be hypothesized that the dysfunctional immunological milieu prevalent in autoimmune disease favors adaptive improvement of antibody catalytic activities over the course of adaptive B cell differentiation. Autoimmune B cells display a lowered threshold to antigenic stimulation.^{27,28} Despite reductions in BCR occupancy due to antigen hydrolysis, therefore, productive signal transduction resulting in clonal proliferation of autoimmune B cells is feasible. The alternative is that the energy liberated at the peptide bond hydrolysis step is itself a stimulatory signal for clonal proliferation of autoimmune B cells.
6. Over its life span in the body, a single catalytic antibody molecule can permanently destroy tens of thousands of target antigen molecules. If catalytic antibodies are sufficiently specific for the target antigen, they will safely clear large amounts of the target. This has inspired our search for specific catalytic antibodies to A β .

Physiological Catalytic Autoantibodies to A β

Michael Sierks at the University of Arizona observed that two recombinant antibody light-chain subunits with VIP-hydrolyzing activity also displayed cross-reactive A β -hydrolyzing activity.⁴⁴ This prompted us to screen monoclonal and polyclonal preparations of intact antibodies for this activity. Of 10 monoclonal IgM antibodies from patients with Waldenström macroglobulinemia with promiscuous proteolytic activity, two also hydrolyzed A β 40 and A β 42.⁴⁵ The catalytic IgMs did not bind A β 40 according to enzyme-linked immunosorbent assay (ELISA) tests. By mass spectrometry of the product peptides, the Lys28-Gly29 and Lys16-Leu17 bonds were identified, respectively, as the major and minor hydrolysis sites. Active site titration using an electrophilic phosphonate diester designed to bind the catalytic site of serine protease enzymes irreversibly indicated the presence of 10.2 catalytic sites/IgM molecule, compared to the theoretical value of 10 antigen-combining sites.⁴⁵ The catalytic activity was retained in the Fab fragments of the IgM, and the activity was maintained at constant levels following successive purification steps. These observations indicated that A β hydrolysis is attributable to the IgM.

To assess the potential of physiological A β clearance by catalytic antibodies, we studied polyclonal antibody preparations purified from human sera.⁴⁵ Most IgM preparations from nondemented elderly humans displayed detectable A β 40 hydrolytic activity. The magnitude of A β hydrolysis was variable over two log orders in a group of 25 study subjects, suggesting a polymorphic distribution of the catalytic antibodies. IgG antibodies displayed lesser levels of activity. Antibodies from comparatively young blood donors (<35 years)

displayed significantly lower levels of A β -hydrolytic activity compared to the elderly donors (>70 years), indicating an aging-induced synthesis of the catalytic antibodies. No hydrolysis of irrelevant polypeptides by the IgMs was evident. It may be concluded that A β 40-specific IgMs are responsible for the catalytic activity.

The concentrations of A β 40 and A β 42 in blood are ≈ 0.2 and ≈ 0.05 ng/mL, respectively.⁴ If IgM present in peripheral blood is assumed to express catalytic activity *in vivo* at the average observed level, >90% of blood-borne A β 40 will be hydrolyzed after 5 days (corresponding to the half-life of IgM in blood; see ref. 45 for details). At concentrations of the IgM displaying readily detectable A β hydrolysis, no A β binding was evident. This suggests that the IgM autoantibodies with catalytic activity hold superior potential for inactivating A β compared to any A β -binding IgMs present in

the blood. Peripheral and brain A β peptide levels are maintained in a state of equilibrium by means of A β transport mechanisms operative at the BBB (Fig 1). Catalytic antibodies can be expected to clear large amounts of peripheral A β . According to the "peripheral drainage hypothesis," autoantibodies that catalyze hydrolysis of peripheral A β may induce depletion of the brain peptide stores.

IgM-class antibodies are large molecules and cross the BBB only in small amounts. However, the brain is an immunocompetent organ, and B cells within the brain hold the potential of producing catalytic antibodies. IgM concentrations in the cerebrospinal fluid (CSF) of nondemented humans and AD patients are in the μ g/mL range.⁴⁶ If the IgMs found in the brain express sufficient catalytic activity, they can directly hydrolyze and clear A β plaques and toxic oligomers within the brain. The catalytic IgMs described above protected a

FIG. 2. Identification and structure of amyloid β peptide (A β)-hydrolyzing immunoglobulins variable (IgV) clones. **(A)** Structure of biotinylated electrophilic A β 1-40 (Bt-E-A β 40). The electrophilic phosphonates placed within the peptide permit specific covalent binding to nucleophilic immunoglobulins (Igs) facilitated by noncovalent epitope recognition. **(B)** Catalytic IgV identification by random screening and covalent selection with Bt-E-A β 40. For IgV screening, 63 IgVs were purified by metal affinity chromatography of periplasmic extracts of bacterial colonies picked randomly from the IgV library. Aliquots of eluates (75 μ L) obtained by metal affinity chromatography of the periplasmic extracts (total eluate volume 0.9 mL) were tested for A β hydrolyzing activity using ¹²⁵I-A β 40 substrate (0.1 nM; 18 h incubation). For covalent selection, the IgV-phage library was treated with Bt-E-A β 40 (2 μ M), covalently bound phages were captured using immobilized anti-biotin antibody, non-covalently bound phage IgVs were removed by treatment with excess A β 40, and the covalent phage IgV immune complexes were recovered by acid disruption of the biotin-anti-biotin antibody complexes (designated covalently selected IgVs). Hydrolysis of ¹²⁵I-A β 40 (0.1 nM) by IgVs was measured using ¹²⁵I-A β 40 substrate (0.1 nM; 18 h incubation). Each point represents an individual IgV clone. **(C)** Schematic representation of IgV_{L2}-t 2E6 and IgV_L-t' 5D3 structures. scFv-t, V_L and V_H domains connected by a peptide linker with t, the His6/c-myc tag located at the carboxyl terminus; IgV_{L2}-t 2E6, a heterodimer of two V_L domains with the linker and t; IgV_L-t' 5D3, a single domain V_L with t' at the carboxyl terminus, corresponding to the peptide linker, a short peptide region in place of the V_H domain and the t tag. Data are from ref. 47.

neuronal cell line from the toxic effect of oligomeric A β . Formation of A β oligomers and fibrillar structures was inhibited potently by a catalytic IgM antibody.⁴⁵ If the catalytic IgMs are present in the brain in sufficient amounts, therefore, they may play a physiological role in clearing A β and alleviating its harmful effects on neuronal function.

We interpret the aging-induced production of catalytic antibodies to A β as a compensatory effort mounted by the immune system that counters the pathology associated with A β accumulation in the brain. Understanding homeostatic processes has historically provided valuable clues to development of engineered drugs for intractable diseases. The following section describes our attempt to identify catalytic antibodies for therapy of AD.

Engineering Efficient A β -Hydrolyzing Antibodies

Recombinant IgVs

Polyclonal antibody preparations are mixtures of individual antibodies with diverse levels of catalytic activity. The expressed antibody repertoire in humans can be as large as 10^{10-11} molecules, and modern screening and selection techniques permit identification of rare antibodies from

libraries consisting of human immunoglobulin V domains (IgVs). We searched for A β -hydrolyzing recombinant IgVs in a library composed of $\approx 10^7$ clones from humans with systemic lupus erythematosus, an autoimmune disease associated with enhanced production of catalytic antibodies.⁴⁷ The majority of the clones in the library are single chain Fv (scFv) molecules with a scaffold that mimics the V domain organization of physiological human antibodies responsible for specific antigen recognition (V_L -Li- V_H -t, where Li denotes a 16-residue linker peptide that joins the V_L domain carboxyl terminus to the V_H domain amino terminus, and t denotes a 26-residue carboxy-terminal peptide containing the *c-myc* peptide and His6 tags that facilitate identification and purification of the scFv molecules).³⁵ A minority of clones possess unusual IgV structures generated by DNA manipulation errors that inevitably accompany repeated nucleic acid replication and cloning cycles over the course of library construction.

By random screening and covalent phage-IgV selection using an A β 40 analog containing electrophilic phosphonates at the side chains of Lys16 and Lys25 (Bt-E-A β 40; Fig. 2A), we isolated rare IgV clones capable of hydrolyzing A β 40 rapidly (Fig. 2B). To rule out sample preparation artifacts, the

FIG. 3. Immunoglobulins variable (IgV) catalytic properties. (A) Cleavage and noncovalent recognition sites. Cleavage sites in A β 40 (indicated with black arrows) or A β 42 (white arrows) were determined by matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF) mass spectrometric analysis of nonradiolabeled synthetic A β peptides (100 μM) incubated with IgV_L-t 2E6 (1 μM ; 89 h). Bigger and smaller arrows denote, respectively, the major and minor cleavage sites in individual peptides. The noncovalent binding determinant for IgV_L-t 2E6 (indicated with a green horizontal bar) was determined by screening synthetic A β peptide fragments for the ability to inhibit IgV-mediated ^{125}I -A β 40 hydrolysis competitively. (B) Inhibition of IgV_L-t 2E6 and IgV_L-t' 5D3 catalyzed ^{125}I -A β 40 hydrolysis by E-hapten-1. IgV_L-t 2E6 (0.55 $\mu\text{g/mL}$) or IgV_L-t' 5D3 (0.075 $\mu\text{g/mL}$) treated with E-hapten-1 (R1 = benzyloxycarbonyl, R2 = phenyl) for 2 h were allowed to hydrolyze ^{125}I -A β 40 as in Fig. 2B. (Inset) Streptavidin-peroxidase stained blots of sodium dodecyl sulfate (SDS)-gels showing IgV_L-t 2E6 (20 $\mu\text{g/mL}$) treated for 18 h with 0.1 mM Bt-E-hapten-1 (R1 = biotinamidohexanoyl, R2 = C₆H₅; lane 2) or poorly electrophilic control hapten-2 (R1 = biotinamidohexanoyl, R2 = H; lane 3). Lane 1, IgV_L-t 2E6 stained with silver. (C) Putative nucleophilic triad in the IgV_L-t deduced by molecular modeling. The amino acid triad contains an H-bonded network that can impart nucleophilic reactivity to the Ser residue. Data are from ref. 47.

phagemid DNA encoding one of the high-activity IgV clones (2E6) was prepared and re-expressed in 15 individual bacterial colonies. IgV 2E6 from all 15 individual colonies expressed robust A β 40-hydrolyzing activity. No activity was observed in empty vector control extract devoid of an IgV insert.

To determine their structure, the cDNAs of four IgVs distinguished by high-level A β 40-hydrolyzing activity were sequenced. None of the catalytic IgVs contains the archetypal V_L-V_H paired structure of physiological Igs. IgV 2E6 is a heterodimer of two different V_L domains with the intervening linker peptide and the expected carboxy-terminal tag (designated IgV_{L2-t}; Fig. 2C). The remaining catalytic IgVs are single-domain V_L clones with unexpected carboxy-terminal polypeptide segments (designated IgV_{L-t'} clones). The carboxy-terminal polypeptide region *t'* consists of (1) the expected linker peptide, (2) a 15- to 28-residue aberrant peptide in place of the customary V_H domain composed of $\approx$ 115 residues, and (3) the expected 26-residue peptide containing *c-myc* and His6 tags (Fig. 2C). Sequencing of 26 randomly picked clones from the library indicated that only a minority are IgV_{L2-t} (12.5%) or IgV_{L-t'} (4.2%) structures. The structure of the IgVs was also confirmed by mass determination of the highly purified proteins, tryptic digestion and mass spectroscopic analysis of the peptides.⁴⁷

The cumulative probability of identifying four high-activity A β 40-hydrolyzing clones from the library with the rare IgV structures in Fig. 2C by random chance alone is very small ($p = 0.92 \times 10^{-5}$). We generated four repaired versions of a highly catalytic IgV_{L-t'} clone (clone 5D3) by insertion of

the missing V_H residues 8–115 into the molecule (Kabat numbering). This yielded scFv clones mimicking the combining sites of physiological Igs. All of the scFv mimetics expressed A β 40-hydrolyzing activity lower than the parent IgV_{L-t'} by $\approx$ 2 orders of magnitude. Partnering of the single V_L domain of the IgV_{L-t'} with full-length V_H domains, therefore, suppresses the catalytic activity. The rate of A β hydrolysis by the IgVs was 3–4 orders of magnitude greater than the naturally occurring IgMs described in the preceding section. Taken together, these observations indicate that the nonphysiological IgV clones identified serendipitously in our studies can express A β -hydrolyzing activity beyond the range observed for natural Igs.

Catalytic properties

Mass spectrometry of the A β fragments produced by the catalytic IgVs indicated that the major hydrolysis site was the His14–Gln15 bond, with smaller levels of hydrolysis occurring at the Gln15–Lys16, Phe20–Ala21, Lys27–Gly28, and Gly28–Ala29 bonds (Fig. 3A).⁴⁷ The IgVs did not hydrolyze irrelevant proteins and model protease substrates customarily employed to monitor catalyst specificity, including proteins containing the His–Gln bond. There is no evidence, therefore, for IgV-catalyzed hydrolysis of proteins other than A β , suggesting the feasibility of specific A β clearance with little or no damage to other proteins. Synthetic A β fragments corresponding to residues 29–40 inhibited 2E6-catalyzed ¹²⁵I-A β 40 hydrolysis as potently as full-length A β 40, while A β 1–17 encompassing the major His14–Gln15 scissile bond did not. This

FIG. 4. Phylogenetic origin of antibody catalysis. The structure of highly catalytic immunoglobulins variable (IgVs) is reminiscent of primordial antibodies, suggesting that catalysis is a primitive function that may have developed prior to the evolution of adaptive immunity mechanisms responsible for the high affinity antigen binding capability of modern antibodies. Efficient catalysts could potentially be obtained from primordial antibody libraries or by reverse-engineering of modern antibodies to mimic primordial antibodies. IgNAR, immunoglobulin new antigen receptor.

indicates that IgV specificity derives mainly from noncovalent recognition of the carboxy-terminal A β region remote from the major scissile bond. Recognition of His14–Gln15 dipeptide unit by the IgV in the transition state of the reaction is necessary for catalytic acceleration of peptide bond hydrolysis. However, as most other scissile bonds of A β do not contain His and Gln residues, the recognition of unique amino acid side chains at the peptide bond transition state must be a lesser contributory factor.

Consistent with the serine protease-like mechanism of previously described catalytic antibodies, the IgVs were bound irreversibly by the biotinylated electrophilic phosphonate diester Bt-E-hapten-1 (Fig. 3B). This probe reacts covalently with the nucleophilic antibody sites responsible for attacking the electrophilic carbonyl of peptide bonds in the initial step of proteolysis.³⁵ The poorly electrophilic control hapten-2 did not form detectable covalent adducts with the IgVs. E-hapten-1 also inhibited ¹²⁵I-A β 40 hydrolysis by the IgVs (Fig. 3B). A molecular model of catalytic IgV_{L2-t} 2E6 identified the potential catalytic site composed of the Ser76–Arg18–Asp17 residues⁴⁷ (Fig. 3C).

The limited efficacy and side effects of a reversibly binding anti-A β monoclonal IgG in patients with mild-to-moderate AD has highlighted the importance of searching for safer and more effective immunotherapeutic reagents.¹⁹ Large quantities of stoichiometrically binding monoclonal IgGs are usually required for immunotherapy. Catalytic Igs hold the potential of clearing A β efficiently by virtue of the specific A β -degrading activity. For example, from its turnover number determined at excess A β concentration, a single IgV_{L-t'} 5D3 molecule will degrade 4320 A β molecules in 3 days.⁴⁷ The rate of catalysis is comparable to that of neprilysin, an enzyme that has received attention as a potential A β -clearing drug.⁴⁸ In unpublished studies, we observed that minimizing conformational perturbations of the IgVs during purification improves the catalysis rate by another order of magnitude. The IgVs are also unique by virtue of their specificity for A β . Neprilysin and other A β -hydrolyzing enzymes, in contrast, hydrolyze irrelevant polypeptides.

A β -binding IgGs are described to induce inflammation and vascular microhemorrhages.^{25,26} The underlying mechanisms are, respectively, immune complex-stimulated release of microglial inflammatory mediators and IgG-stimulated A β deposition in cerebral blood vessels. The IgVs failed to bind immobilized A β 40 in ELISA tests, indicating their inability to form stable immune complexes. Moreover, they degrade A β permanently, minimizing the risk of A β re-deposition in the vascular walls. Taken together, the observed properties of the IgVs support the idea that they can be developed to specifically and efficiently clear A β with minimal side effects.

Phylogenic origin of antibody catalysis

Modern adaptive immunity is characterized by high-affinity antibody binding to antigens. The V_L and V_H domains of higher organisms express substantial sequence identity with each other. They have likely evolved by duplication and sequence diversification of a common primordial gene encoding the Ig fold. The structure of the best antibody catalysts is reminiscent of primordial antibodies.

Jawed fish contain the earliest known immune system.⁴⁹ Their immune system produces a functional homolog of the single-domain catalytic IgVs, i.e., a single V_H domain thought to bind antigen in its unpaired state. Jawed fish also produce IgMs, but not other classes of antibodies. In higher organisms, IgMs are the first class of antibodies produced by B cells, with other antibody classes appearing at later stages of the adaptive immune response. IgMs from humans and mice consistently express catalytic activities superior to class-switched IgG antibodies.⁵⁰ These arguments suggest that catalysis is a primitive function that may have developed prior to the evolution of binding activity (Fig. 4). For the antibody engineer, these ideas present novel opportunities, because primordial antibodies may be a rich source of efficient catalysts.

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