Constant Domain-regulated Antibody Catalysis*

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Background: Some antibody variable domains express nucleophilic catalytic sites.

Results: The same variable domains displayed superior catalysis when expressed in the IgM compared with the IgG constant domain scaffold; all monoclonal IgMs were catalytic, and polyclonal IgM was more catalytic than IgG.

Conclusion: The constant domains regulate variable domain catalysis.

Significance: Catalysis may be a first line immune function expressed prior to adaptive antibody induction.

Some antibodies contain variable (V) domain catalytic sites. We report the superior amide and peptide bond-hydrolyzing activity of the same heavy and light chain V domains expressed in the IgM constant domain scaffold compared with the IgG scaffold. The superior catalytic activity of recombinant IgM was evident using two substrates, a small model peptide that is hydrolyzed without involvement of high affinity epitope binding, and HIV gp120, which is recognized specifically by noncovalent means prior to the hydrolytic reaction. The catalytic activity was inhibited by an electrophilic phosphonate diester, consistent with a nucleophilic catalytic mechanism. All 13 monoclonal IgMs tested displayed robust hydrolytic activities varying over a 91-fold range, consistent with expression of the catalytic functions at distinct levels by different V domains. The catalytic activity of polyclonal IgM was superior to polyclonal IgG from the same sera, indicating that on average IgMs express the catalytic function at levels greater than IgGs. The findings indicate a favorable effect of the remote IgM constant domain scaffold on the integrity of the V-domain catalytic site and provide a structural basis for conceiving antibody catalysis as a first line immune function expressed at high levels prior to development of mature IgG class antibodies.

Antibodies (immunoglobulins) are generated by linking the constant (C)4-domain genes encoding the heavy chain (μ, δ, γ, α, ε) or light chain (κ, λ) to the variable (V)-domain genes, that in turn are generated by rearrangement of about 500 V, (D) and J germ line genes. The C-domains define the antibody class and subclass. The paired heavy and light chain V-domains (VH, VL domains) contain the antigen combining site. The C-domains contain the “effector” sites mediating complement fixation and Fc receptor activation. Despite their spatial separation, the V- and C-domain sites display inter-dependent functional relationships. Antigen binding at the V-domains stimulates complement and Fc receptor binding by the C-domain sites (1). Noncovalent antigen binding to the V domains generally occurs without dependence on the C-domains, but subtle effects of the IgG C-domains are documented (for review, see Ref. 2). Placing the same VH-VL domain pair into the differing C-domain scaffold of various IgG subclasses can induce alterations of antigen binding affinity and fine specificity (3). The V-domains are subject to conformational transitions upon linkage to different constant domains. Identical VH-VL domains cloned into different IgG subclasses are bound nonequivalently by an anti-idiotypic antibody directed to V-domain epitopes (3). Similarly, the circular dichroism spectra of the differing IgG isotypes containing the same V-domains in the presence of antigen are nonidentical (4).

Antibodies are initially expressed on the B cell surface as IgM or IgD B cell receptors (BCRs) complexed to signal transducing proteins. The V-(D)-J gene rearrangement and combinatorial VL-VH pairing events produce a diverse innate repertoire composed of >1012 secreted IgMs that is shaped by various epigenetic factors and contact with self-antigens (5, 6). The V- and C-domains go through programmed structural changes during B cell differentiation. Accumulation of V-domain somatic mutations in the immunogen-driven differentiation phase improves the immunogen binding affinity. C-domain class switching required for production of IgGs and IgAs can occur in the absence of immunogen, but this process often takes place

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This article contains supplemental Fig. S1, Tables S1–S3, and additional references.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBankTM/EBI Data Bank with accession number(s) AF329462 and JX390613.

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4 The abbreviations used are: C-domain, constant domain; AMC, 7-aminomethylcoumarin; BCR, B cell receptor; gp120, glycoprotein 120; scFv, single chain Fv; V-domain, variable domain; VH, heavy chain variable domain; VL, light chain variable domain; VIP, vasoactive intestinal peptide; WM, Waldenström macroglobulinemia.
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Polypeptides and Hapten—The fluorogenic peptide Glu-Ala-Arg-7-amino-4-methylcoumarin (AMC) was from Peptides International (Louisville, KY). Recombinant gp120 (MN strain) from Immunodiagnostics (Woburn, MA) was linked to biotin (1.8 mol of biotin/mol) at Lys residues (22). Synthetic nonelectrophilic peptide 421–436 conjugated to BSA (6 mol of peptide/mol) and electrophilic E-421–433 with biotin at the N terminus contained the consensus subtype B gp120 421–433 residues (7, 23). Control biotin-E-VIP was prepared as in Ref. 24. The chemical identity of all peptides was verified by electrospray ionization mass spectrometry. Electrophilic haptents (E-Hapten-1, E-Hapten-3) and the nonelectrophilic hapten (E-Hapten-2) were synthesized as in Refs. 19, 25. E-Hapten-1 and E-Hapten-3 are diphenylphosphonate esters reactive with nucleophilic sites (7, 26). E-Hapten-1 and E-Hapten-2 contain biotin to permit detection of adducts by electrophoresis. Hapten-2 is the unesterified phosphonic acid analog of E-Hapten-1 devoid of electrophilic reactivity.

Antibodies—The panel of monoclonal IgMs from 13 patients with Waldenström macroglobulinemia (WM; lymphoplasmacytic lymphoma) is described in Ref. 19 (identification codes 1718, 1801, 1805, 1808–1810, 1812–1814, 1816–1819, serum IgM 9.2–55.7 mg/ml). Polyclonal IgM and IgG pools were purified from the sera of 34 humans without evidence of disease by affinity chromatography on immobilized anti-human IgM and protein G columns, respectively (17 females, 17 males; age 17–65; identification codes 679, 681–689, and 2058–2081, Gulf Coast Blood Bank) (19, 27). Protein concentrations were determined using bicinchoninic acid. Purity was evaluated by SDS-gel electrophoresis under nonreducing and reducing conditions followed by staining with Coomassie Blue or specific peroxidase-conjugated antibodies to the λ, γ, and μ subunits (19). All protein bands present in the IgM and IgG preparations were stainable with these antibodies. scFv JL427 was isolated from a phage library from humans without HIV infection by binding to immobilized gp120, expressed in bacteria, and purified by metal-affinity chromatography to electrophoretic homogeneity (28). A molecular model of the scFv was constructed using a homology-based method (WAM). Potential nucleophilic sites were located using the previously described algorithm for identifying enzyme-like amino acid triads and diad (21). To construct full-length antibodies, the Vλ and Vμ domain cDNA (corresponding to amino acid residues 1–128 and 1–127, respectively, IMGT numbering) were amplified by the polymerase chain reaction and expressed linked to the human IgG1 constant domain scaffold as in Refs. 29, 30 except that the Vλ domain was cloned on the 5’ side of the λ constant domain gene (corresponding to JGLC domain amino acids 1–106 in the IMGT system) in the pLCH-huCA vector using the BglII/NotI site. Similar procedures were used to construct the JL427 μ chain. The leader-Vμ cDNA excised from the JL427 pHCh-huCy1 vector using Nhel/HindIII was cloned on the 5’ side of the μ constant domain genes (corresponding to IGHM CH1 residues 1–104, CH2 residues 1–100, CH3 residues 1–106, CH4 residues 1–131) in pHC-huChμ vector via the Nhel/HindIII sites. To accommodate insert cloning and vector shuffling procedures the following antibody residues were mutated: the two N-terminal Vμ residues from QV to EF, the μ CH1 residue 3 from A to L, the γ1 CH1 residue 3 from T to F, and the CA residues 2–4 from QPK to RTA. PCR primers are listed in supplemental Table S1. Dideoxy nucleotide sequencing of the IgG and IgM V-domains in both directions yielded identical
sequences that matched the parental V_{L}^{-} and V_{H}^{-}-domain sequences. Full-length IgG and IgM were obtained in the supernatants of stable N50 cell lines coexpressing the light and heavy chain vectors grown at a density of 1.6 × 10^{4} cells/ml in CEL-Line flasks (Wilson Wolf Corp., New Brighton, MN) (30). IgG-depleated FBS was used for cell culture. The concentration of bovine IgM in FBS is insignificant. Secreted IgG and IgM were measured by capture ELISA on wells coated with anti-λ antibody (200 ng/well; Sigma-Aldrich) and peroxidase-conjugated goat anti-IgG1 or anti-IgM antibodies (1:1000; Sigma-Aldrich) with IgG1 and IgM as standards. Expression levels were ~3.3 mg of IgM/liter of IgM and 4.0 mg/liter IgG. The antibodies were purified from 10-fold concentrated tissue culture supernatants (Centriprep YM10; Millipore) by chromatography on immobilized anti-IgG1 antibody and protein G columns as before. Further IgM size exclusion chromatography was on a Superose-6 FPLC column in 50 mM Tris-HCl, pH 7.8, 0.1 M glycine, 0.1 M NaCl, 0.1 mM CHAPS (0.4 ml/min) or the same buffer containing the denaturant 6 M guanidine-HCl adjusted to pH 6.5 with HCl (0.13 mM) (19). The retention volumes of the pentamer and monomer IgM fractions recovered from the nondenaturing column were 9.0 ml and 15.8 ml, respectively, and from the denaturing column, 5.7 ml and 8.8 ml, respectively. The shorter retention times in the denaturing column are consistent with lesser permeation of the gel pores by nonglobular unfolded proteins. Column calibration was with thyroglobulin (660 kDa), IgG (150 kDa), and albumin (67 kDa) (Sigma-Aldrich).

**Binding and Catalysis Assays—scFv JL427 binding to the immobilized BSA-conjugated gp120 peptide 421–436 (230 ng/well) was measured using an antibody to the c-myb tag located at the scFv C terminus (28). scFv binding to electrophilic probes was determined by SDS-electrophoresis using boiled reaction mixtures followed by staining of blots with peroxidase-conjugated streptavidin (7). Hydrolysis of the amide bond linking AMC to the C-terminal Arg in Glu-Ala-Arg-AMC (7) was measured by fluorometry (Fig. 1A). The IgMs contain an identical C-domain scaffold. Yet, the catalytic activity of individual IgMs varied >91-fold, indicating that the catalytic activity is a V-domain function. This is consistent with the finding of catalysis by the Fab fragment of an IgM antibody (19). In addition, previous studies have identified nucleophilic sites in the C-domain-free V-domains with varying levels of catalytic activity, including recombinant scFvs (V_{L}^- -linker-V_{H}^- constructs) (7) and isolated V_{L}^- domains (31). Fig. 1B reports the comparative Glu-Ala-Arg-AMC hydrolytic rates of the monoclonal IgMs with the highest and lowest activities along with pooled polyclonal IgM and IgG from the same sera. The hydrolytic rates for serum IgM and IgG from individual human donors have been reported previously (14, 19, 27). The polyclonal IgG pool displayed detectable but low catalytic activity (0.33 μM substrate/μg IgG at 21 h, the final observation point). Even the least catalytic monoclonal IgM (1801) hydrolyzed Glu-Ala-Arg-AMC more rapidly than polyclonal IgG (by 18-fold). The hydrolytic rate of polyclonal IgM was 939-fold superior to polyclonal IgG.

**Source V Domain Properties**—The V_{L}^- -V_{H}^- domain pair from scFv JL427 was used to prepare IgM and IgG as described in the next section. The scFv was isolated by fractionating a human scFv library displayed on phages using immobilized HIV gp120 as the selection reagent. The JL427 V domains contain a large number of somatic mutations (supplemental Table S2; GenBank accession number AF329462). Fig. 24 shows the noncovalent binding of scFv JL427 to synthetic peptide 421–436, similar to the specificity of other gp120-binding scFv clones isolated from this library (28). The scFv formed 32-kDa covalent adducts with the electrophilic analog of peptide 421–433

**RESULTS**

**Monoclonal IgM Catalytic Activity**—Hydrolysis of the amide bond linking the fluorophore AMC group to small model peptide substrates is a convenient surrogate for peptide bond hydrolysis by antibodies. The reaction occurs preferentially on the C-terminal side of Arg/Lys residues and does not require high affinity binding to an antigenic epitope (19). All 13 monoclonal human IgMs from patients with WM hydrolyzed Glu-Ala-Arg-AMC detectably (Fig. 1A). The IgMs contain an identical C-domain scaffold. Yet, the catalytic activity of individual IgMs varied >91-fold, indicating that the catalytic activity is a V-domain function. This is consistent with the finding of catalysis by the Fab fragment of an IgM antibody (19). In addition, previous studies have identified nucleophilic sites in the C-domain-free V-domains with varying levels of catalytic activity, including recombinant scFvs (V_{L}^- -linker-V_{H}^- constructs) (7) and isolated V_{L}^- domains (31). Fig. 1B reports the comparative Glu-Ala-Arg-AMC hydrolytic rates of the monoclonal IgMs with the highest and lowest activities along with pooled polyclonal IgM and IgG from the same sera. The hydrolytic rates for serum IgM and IgG from individual human donors have been reported previously (14, 19, 27). The polyclonal IgG pool displayed detectable but low catalytic activity (0.33 μM substrate/μg IgG at 21 h, the final observation point). Even the least catalytic monoclonal IgM (1801) hydrolyzed Glu-Ala-Arg-AMC more rapidly than polyclonal IgG (by 18-fold). The hydrolytic rate of polyclonal IgM was 939-fold superior to polyclonal IgG.

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was permissive for forming candidate nucleophilic sites (sup-33). Molecular modeling suggested that the scFv conformation hydrogen bonding between amino acid triads and diads (32, nucleophilic sites are formed by conformation-dependent tively). The data suggest a nucleophilic site that reacts with the E-Hapten adducts were visible at a 10-fold higher concentration of supernatants of NS0 lymphoid cells coexpressing vectors containing the same V domains from scFv JL427 into the full-length IgM scaffold. The antibodies were obtained from culture fermentations of N50 lymphoid cells coexpressing vectors containing the V-domain cDNA cloned adjacent to the μ, λ or γ1 C-domain genes. The cells express the J chain needed for assembling pentameric IgM (900 kDa) constitutively. The IgM and IgG preparations purified using immobilized anti-IgM antibody and protein G, respectively, contained heavy and light chain subunit bands with the anticipated mass in reducing SDS-electrophoresis gels (Fig. 3A). A major, correctly assembled 150-kDa band along with two incompletely assembled oligomer bands were evident in the IgG preparation in a nonreducing SDS-gel (Fig. 3A, lanes 3 and 4). Gel filtration of the IgM in a denaturing solvent (6 M guanidine hydrochloride) indicated a majority species with nominal mass of pentamer IgM (52% of recovered protein; mass measured by comparison with marker proteins, 1070 kDa), along with a minority monomer IgM species (29%; nominal mass, 195 kDa) and free subunits (Fig. 3B).}

FIGURE 3. IgM and IgG electrophoresis and gel filtration. A, SDS-electrophoresis gels of anti-IgM-purified IgM JL427 and protein G-purified IgG JL427. Lanes 1 and 2, reducing SDS-gels of the IgG stained with Coomassie Blue or anti-γ/λ antibodies, respectively. Lanes 3 and 4, nonreducing SDS-gels of the IgG stained with Coomassie Blue or anti-γ/λ antibodies, respectively. Lanes 5 and 6, reducing SDS-gels of the IgM stained with silver or anti-μ/λ antibodies, respectively. Band masses in kDa are indicated. Bands at 132 and 111 kDa in the nonreducing IgG lane are incompletely assembled oligomers. B, IgM JL427 gel filtration. The IgM purified by anti-IgM antibody chromatography was subjected to denaturing Superose 6 gel filtration in 6 M guanidine-HCl. The yellow pentamer fraction was renatured and tested for catalytic activity. Arrows indicates elution of the marker proteins thyroglobulin, IgG3, and BSA.

(O-421–433) but not an irrelevant electrophilic peptide (E-VIP) (Fig. 2, B and C). The adducts were stable to denaturing conditions that dissociate noncovalent binding (boiling, SDS treatment), indicating a covalent nucleophile-electrophile reaction. No scFv adducts were formed at an equivalent electrophilic hapten phosphonate diester concentration (E-Hapten-1). The adducts were visible at a 10-fold higher concentration of E-Hapten-1 but not the control hapten phosphonic acid 2 devoid of electrophilic reactivity (Fig. 2D, lanes 1 and 2, respectively). The data suggest a nucleophilic site that reacts with the electrophile guided by noncovalent epitope binding. The nucleophilic sites are formed by conformation-dependent hydrogen bonding between amino acid triads and diads (32, 33). Molecular modeling suggested that the scFv conformation was permissive for forming candidate nucleophilic sites (sup-33mental Table S3).

Catalytic Activity of IgM and IgG JL427—To eliminate V-domain differences as a contributory factor in catalysis, we cloned the same V domains from scFv JL427 into the full-length IgM and IgG scaffolds. The antibodies were obtained from culture supernatants of NS0 lymphoid cells coexpressing vectors containing the V-domain cDNA cloned adjacent to the μ, λ or γ1 C-domain genes. The cells express the J chain needed for assembling pentameric IgM (900 kDa) constitutively. The IgM and IgG preparations purified using immobilized anti-IgM antibody and protein G, respectively, contained heavy and light chain subunit bands with the anticipated mass in reducing SDS-electrophoresis gels (Fig. 3A). A major, correctly assembled 150-kDa band along with two incompletely assembled oligomer bands were evident in the IgG preparation in a nonreducing SDS-gel (Fig. 3A, lanes 3 and 4). Gel filtration of the IgM in a denaturing solvent (6 M guanidine hydrochloride) indicated a majority species with nominal mass of pentamer IgM (52% of recovered protein; mass measured by comparison with marker proteins, 1070 kDa), along with a minority monomer IgM species (29%; nominal mass, 195 kDa) and free subunits (Fig. 3B). scFv JL427 and its IgG counterpart hydrolyzed Glu-Ala-Arg-AMC at detectable but low levels (Fig. 4A). The hydrolytic activity of IgM JL427 was superior to the scFv and IgG (Fig. 4A, by 740-fold and 202-fold, respectively). Ten repeat assays using two independent IgM preparations and four assays using three IgG preparations confirmed consistently more rapid catalysis by the IgM (expressed per mole of antibody, by 328 ± 218-fold, p < 0.0001, unpaired t test). To preclude noncovalently associ-
ated trace contaminants, the IgM purified by affinity chromatography on immobilized anti-IgM antibody, IgM, renatured pentamer is the yellow fraction from Fig. 3B (retention volume 5.6 – 6.4 ml) obtained by gel filtration in denaturant and renatured by dialysis against 50 mM Tris-HCl, 0.1 mM glycine, 0.15 mM NaCl, 0.1 mM CHAPS. IgG and scFv were purified using immobilized protein G and nickel, respectively. Data are means ± S.D. of three replicates expressed as μM substrate hydrolyzed/μM antibody. Reaction conditions: IgM, native 11 nM; IgM, renatured pentamer 11 nM; IgG, 180 nM; scFv, 350 nM; Glu-Ala-Arg-AMC, 400 μM. B, progress curves of Glu-Ala-Arg-AMC hydrolysis by the pentamer IgM (retention volume 8.6 – 9.8 ml) and monomer IgM (retention volume 15.4 – 16.2 ml) purified by gel filtration in nondenaturing solvent. IgG data are from A. IgM purified using immobilized anti-IgM was loaded on the gel filtration column. Data are means ± S.D. of three replicates expressed as μM substrate hydrolyzed/μM combining sites (pentamer and monomer IgM, 10 and 2 combining sites, respectively). Reaction conditions: IgM pentamer, 0.7 nM; IgM monomer, 7 nM; Glu-Ala-Arg-AMC, 300 μM. C, inhibition of IgM JL427 hydrolytic activity by serine protease inhibitor E-Hapten-3. Progress curves show Glu-Ala-Arg-AMC hydrolysis by IgM in the presence or absence of E-Hapten-3 (means ± S.D. of three replicates). Reaction conditions: IgM, 8.8 nM; Glu-Ala-Arg-AMC, 400 μM; E-Hapten-3, 100 μM. D, saturation kinetics of Glu-Ala-Arg-AMC hydrolysis by the IgM and IgG. Initial velocities V<sub>i</sub> fitted to the Michaelis-Menten equation V = (k<sub>cat</sub>[Ab][S])/(K<sub>m</sub> + [S]) by nonlinear regression. [Ab], antibody concentration; [S], initial substrate concentration. In the table, the values of k<sub>cat</sub> outside and inside the parentheses correspond, respectively, to moles of substrate hydrolyzed/mole of antibody per min or moles of substrate hydrolyzed/mole of antibody combining sites/min (10 and 2 combining sites in IgM and IgG, respectively). Reaction conditions: IgM, 18 nM, incubated for 18.5 h; IgG, 133 nM, incubated for 28.5 h.

FIGURE 4. Model peptide hydrolytic properties of IgM, IgG, and scFv JL427. A, time-dependent hydrolysis of Glu-Ala-Arg-AMC. IgM, native is the IgM purified by affinity chromatography on immobilized anti-IgM antibody. IgM, renatured pentamer is the yellow fraction from Fig. 3B (retention volume 5.6 – 6.4 ml) obtained by gel filtration in denaturant and renatured by dialysis against 50 mM Tris-HCl, 0.1 mM glycine, 0.15 mM NaCl, 0.1 mM CHAPS. IgG and scFv were purified using immobilized protein G and nickel, respectively. Data are means ± S.D. of three replicates expressed as μM substrate hydrolyzed/μM antibody.
valence for IgM is 27-fold greater than IgG. As the V-domains of the IgM and IgG are identical, the superior catalytic activity of IgM is attributable to a favorable C-domain effect on V-domain catalysis.

We also measured the hydrolysis of biotinylated gp120 to verify hydrolysis of true peptide bonds. Because the gp120 is available only in limited quantities, the assays were conducted at a nonsaturating gp120 concentration (100 nM). No hydrolytic activity of the IgM is detectable using Glu-Ala-Arg-AMC at this substrate concentration. scFv JL427 V-domains expressed in the IgM scaffold were noncovalently bound to gp120 and displayed catalytic activity for gp120 hydrolysis after 24 h.

**DISCUSSION**

Individual antibody species within a given antibody class can express varying catalytic activities because of their differing V-domain structures, illustrated by the finding of widely divergent activities of monoclonal IgMs with identical C-domain structures. Polyclonal antibody studies indicated that the average catalytic activity of IgMs far exceeds that of IgGs (15, 19, 35). IgGs develop from IgMs by a switch of C-domain gene expression first line immune function that is retained upon accumulation of somatic V-domain mutations if unaccompanied by class-switching to IgG. In contrast, IgGs dominate the adaptive, noncovalent binding response to immunogens. Catalytic IgMs may fulfill defense functions if directed to microbes or toxic autoantigens, and pathogenic functions if directed to essential autoantigens.
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cating that more avid noncovalent binding due to differing antibody valence is not a factor (note: multivalent binding of substrates devoid of repeat epitopes, e.g. Glu-Ala-Arg-AMC, is precluded in solution-state assays). Loss of substrate binding affinity (increased $K_m$) is described to improve the catalytic rate constant due to a decrease of the reaction activation energy (36). The IgM C-domains exert a favorable effect on the catalytic rate constant without an alteration of the $K_m$ value, indicating improved catalysis independent of the initial noncovalent binding step. In addition to the model peptide substrate, IgM JL427 hydrolyzed gp120 more efficiently than the IgG containing the same V-domains. The V-domains employed for IgM construction bind the 421–433 gp120 epitope specifically. Catalytic antibodies that hydrolyze gp120 with specificity derived from noncovalent binding to the 421–433 epitope were described previously (19, 34).

Catalysis is a germ line BCR-encoded function that is expressed with no requirement for B cell encounter with an immunogen (12, 13). According to the B cell clonal selection theory, immunogen-BCR binding drives synthesis of antibodies with somatically mutated V-domains. BCR-catalyzed immunogen hydrolysis will cause release of product fragments, depriving B cells of the stimulatory binding signal. Although adaptive selection of sequence-diversified V-domains may well reduce the germ line-encoded catalytic activity, this factor alone does not explain satisfactorily the observation of superior IgM catalysis. IgM JL427 contains V-domains with extensive deviations from their germ line gene sequences due to the V-(D)-J gene rearrangement and somatic mutation processes. The level of somatic sequence deviations is comparable with adaptively generated IgGs (37). Moreover, the V-domains of WM IgMs in Ref. 40, compared with 6.6% VH mutations for 12 IgG from healthy humans (respectively, 8.9 and 11.6 and 0.032 ± 0.003 μM Glu-Ala-Arg-AMC cleaved/μM antibody per h), and all 13 WM IgMs were more hydrolytic than polyclonal IgG. If increasing V-domain mutation is the sole reason for deteriorated IgG catalysis, the WM IgMs and polyclonal IgG should be comparably hydrolytic.

Mutagenesis and crystallography studies have identified V-domain catalytic sites in which a Ser or Tyr side chain nucleophile is activated by H-bonding to a general base (e.g. His, Arg) (8–11). Consistent with the nucleophilic catalytic mechanism, the electrophilic E-Hapten-1 inhibited the hydrolytic activity of IgM JL427. The same probe was previously shown to inhibit other catalytic antibodies irreversibly (19), including antibody V-domains devoid of C-domains (31, 42). We did not identify the IgM catalytic site or determine the mechanistic basis of the C-domain effect on catalysis, but a conceptual framework for further analysis is available. The poorly catalytic scFv JL427 contained a V-domain nucleophilic site based on formation of irreversible complexes with electrophilic probes. A V-domain structural change caused by linkage to the spatially distant IgM C-domains that accelerates a rate-limiting step needed to complete the catalytic cycle will explain the observed improvement of catalysis, e.g. water attack on the acyl-antibody intermediate and product release (supplemental Fig. S1A). Sub-Ångstrom alterations of the catalytic site topography can be induced by remote structural changes in enzymes (43) and antibodies (31). Catalytic sites accomplish nucleophilic attack on peptide bonds, acyl-enzyme hydrolysis, and product release by virtue of precisely positioned functional groups and small conformational rearrangements occurring during the catalytic reaction. For instance, the trypsin Ser nucleophile is deprotonated by the H-bonded His, the proton is donated to the C-terminal substrate leaving fragment, and the same His in an altered orientation deprotonates the water molecule responsible for hydrolysis of the acyl-enzyme intermediate (33). Interfacial bound water has been identified in crystal structures of antigens complexed to high affinity antibody fragments (44), but no structural information is available about limitations in antibody catalysis at the water attack step.

IgMs are found at blood concentrations of $\sim 2 \mu M$ compared with picomolar-nanomolar concentrations of classical proteases (45, 46). Peptide bond hydrolysis often results in inactivation of polypeptides. The gain in biological efficacy due to catalysis can be illustrated from the observed hydrolytic rates of polyclonal IgM from healthy humans at saturating concentrations of gp120 and small peptide substrates (2.1–2.8/min) (19). Large amounts of these antigens will be hydrolyzed over a single half-life of blood-borne IgM ($>9000$ mol of antigen/mg of IgM over 3 days). In comparison, a maximum of 2 mol of antigen is bound per mol of stoichiometric IgGs with noncovalent binding activity. We described antibodies and antibody fragments to HIV gp120 (34), the Staphylococcus aureus virulence factor Efb (20) and the autoantigen amyloid β (47) with neutralizing activity attributable to the catalytic function. The group of Uda and Hifumi has described catalytic antibody fragments to bacterial and viral target antigens that reduce infection in experimental animal models (48, 49). Autoimmune disease is associated with increased catalytic antibodies to autoantigens, and numerous examples of pathogenic catalytic autoantibodies have been reported (50–54; for review see Ref. 55). Conversely, examples of beneficial (physiological) catalytic autoantibodies to toxic
amyloid β aggregates (47) and the coagulation enzyme Factor IX (56) are available. Maintenance of homeostatic levels of antibodies that hydrolyze small model peptides is associated with reduced death in septic shock (57), transplant rejection (58), and autoimmune disease incidence (14, 59). The phenomenon of naturally occurring antibody catalysis was discovered from studies on IgG class autoantibodies (50). Contrary to the assumption that catalysis improves with adaptive maturation of antibodies, our findings suggest that catalysis is a first line defense function of immature, minimally mutated IgM antibodies (Fig. 6). Somatic maturation without class switching is also compatible with expression of catalysis, supporting consideration of the catalytic IgMs with mutated V-domains as functionally important adaptive mediators.

In summary, the findings show that the C-domain scaffold is an important factor influencing expression of V-domain catalytic activity. Future functional studies hold the potential of generating more precise insight to the beneficial and pathogenic functions of catalytic IgMs. In addition, monoclonal and polyclonal catalytic IgMs are of interest as potential therapeutic reagents.

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