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Efficient vasoactive intestinal polypeptide hydrolyzing autoantibody light chains selected by phage display

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Abstract

An immunoglobulin light chain (L chain) library derived from the peripheral blood lymphocytes of a patient with asthma was cloned into a phagemid vector. Phage particles displaying L chains capable of binding vasoactive intestinal polypeptide (VIP) were isolated by affinity chromatography. Two VIP binding L chains were expressed in *Escherichia coli* in soluble form and purified to electrophoretic homogeneity by metal chelating and protein L affinity chromatography. Both L chains catalyzed the hydrolysis of [tyr$^{10\text{-}125}$I]VIP substrate. The catalytic activity eluted at the molecular mass of the monomer form of the L chain (28 kDa) from a gel filtration column. The activity was bound by immobilized anti-κ-chain antibody. A control recombinant L chain displayed no catalytic activity. Hydrolysis of VIP by the catalytic L chains was saturable and consistent with Michaelis-Menten kinetics. The turnover of the L chains was moderate (0.22 and 2.21/min) and their K_m values indicated comparatively high affinity recognition of VIP [111 and 202 nM), producing catalytic efficiencies comparable to or greater than trypsin. Unlike trypsin, the L chains did not display detectable cleavage of casein, suggesting a catalytic activity specialized for VIP. Comparisons of the nucleotide sequences of the L chain cDNA with their putative germ-line counterparts suggested the presence of several replacement mutations in the complementarity determining regions (CDRs). These observations suggest: (a) Retention or acquisition of catalytic activity by the L chains is compatible with affinity maturation of antibodies; and (b) The autoimmune L chain repertoire can serve as a source of substrate-specific and efficient catalysts.

Keywords: Catalytic antibody; Autoantibody; Light chain; Vasoactive intestinal polypeptide; Asthma; Phage display

1. Introduction

Catalysis occurs by stabilization of substrate transition states. It has been commonly assumed, therefore, that immunization with structural analogs of the transition state is necessary to induce catalytic antibody formation (reviewed in Ref. [1]). Contrary to this assumption, polyclonal human autoantibodies are demonstrated to catalyze energetically demanding reactions like peptide and DNA hydrolysis with kinetic efficiencies comparable to those of enzymes [2–7]. Furthermore, mouse strains genetically predisposed to autoimmune disease appear to mount substantially stronger catalytic antibody responses to a transition state analog than control animals [8], supporting the association of catalytic activity with autoimmune reactivity deduced from human studies. The life-times of substrate transition states are exceedingly short, being of the order of bond vibration and rotation frequencies. Sensitization of the immune system by transition states, therefore, is an unlikely mechanism for the formation of catalytic autoantibodies. More plausible explanations for the phenomenon of autoantibody catalysis are: (a) the catalytic activity may be encoded by a germ-line variable region (V-region) gene(s) or developed de novo over the course of affinity maturation of this gene, and (b) Anti-idiotypic antibodies to anti-enzyme autoantibodies found in autoimmune disease may display catalytic activity by mimicking the structural features of the enzyme active site. These possibilities are based on observations of catalysis by antibodies and antibody light chains (L chains) elicited by immunization with the ground state of ordinary antigens [9–11], presence of polyreactive catalytic antibodies in unimmunized donors [12], apparently fortuitous hydrolysis of a substrate unrelated in structure to the immunogen [13] and examples of

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catalysis by anti-idiotypic antibodies to anti-acetylcholinesterase [14] and anti-DNase antibodies [15].

Vasoactive intestinal polypeptide (VIP) is a neuropeptide with potent smooth muscle relaxant, anti-inflammatory and tumor growth promoting effects [16–18]. Polyclonal autoantibodies and their L chain subunits capable of catalyzing the cleavage of VIP have been identified in patients with respiratory disorders [2,3,19,20] and classical autoimmune diseases like systemic lupus erythematosus (S. Paul and coworkers, unpublished results). The aim of the present study was to clone human autoantibody L chains capable of catalyzing the hydrolysis of VIP. This aim is driven by the need for homogeneous human antibody preparations suitable for study of the possible pathogenetic role of VIP antibodies in autoimmune disease, as well the potential utility of the catalytic antibodies as anti-tumor agents. By using phage display methods, two L chains with VIP cleaving activity have been cloned. The catalytic efficiency of the L chains is comparable to trypsin. Unlike trypsin, the L chains are selective for VIP.

2. Materials and methods

2.1. Reagents

Phagemid vector pCANTAB5*his*$_6$ and *Escherichia coli* strains TG1 and HB2151 were from Cambridge Antibody Technologies, Cambridgeshire, UK. Protein L was kindly provided by Dr. L. Björk, University of Lund, Sweden [21]. Synthetic VIP was purchased from Bachem, Torrance, California. Polymerase chain reation (PCR) primers were synthesized and HPLC-purified by National Biosciences, Plymouth, MN. VCSM13 helper phage was from Stratagene, La Jolla, CA. Hybridoma cell line 9E10 producing anti-c-*myc* antibody was from American Type Culture Collection (Rockville, MD), polyethylene glycol (M_r 8000) from Sigma (St. Louis, MO), isopropyl-β-D-thiogalactopyranoside (IPTG) from Clontech (Palo Alto, CA) and resorufin-casein from Boehringer-Mannheim (Indianapolis, IN).

2.2. Recombinant L chains

L chain cDNA was prepared from the peripheral blood lymphocytes of a patient with exercise-induced asthma by the reverse transcriptase-PCR method. κBACK1 primer, κFOR primer and PCR conditions used for cDNA amplification are described in [22]. The PCR products were cloned into the phagemid vector pCANTAB5*his*$_6$, which allows display of L chains on the surface of phage particles or their expression as soluble proteins [23]. Following electroporation of competent *E. coli* TG1 cells with the phagemid DNA, phage particles displaying L chains fused to protein 3 were rescued by superinfection with VCSM13 helper phage, precipitated with polyethylene glycol and subjected to affinity chromatography on a VIP-Sepharose column [22]. Two clones isolated from the pH 5.5 eluate of this column (designated clones hk13 and hk14) were shown to bind VIP by radioimmunoassay and ELISA [24]. These were grown in *E. coli* HB2151 cells and the cultures were induced with IPTG (1 mM) for 24 h to permit secretion of soluble L chains into the supernatant. The expression level of the recombinant L chains in the culture supernatants was ~0.5 mg/liter, estimated by enzyme-linked immunoflow assay for c-*myc* stainable protein [22]. For expression of L chains in the periplasm, induction of the cultures with IPTG was for 3 h. Periplasmic extract preparation was as in [25]. A control catalytic L chain and a non-catalytic L chain from murine hybridoma cell-lines c23.5 and c23.1 [9], respectively, were cloned in pCANTAB5*his*$_6$ vector [25], except that the 5′ back primer used for c23.1 L chain amplification was GTCCTCG-CAACTGC*GGCCCAGCCGGCC*ATGGCC(A/C)A(C/T)A TTGT(A/G/T)CT(G/C)ACCCAGTCTCC (*Sfi*I restriction site is underlined).

2.3. Purification of L chains

The cloned L chains contain a C-terminal hexahistidine tag, permitting their purification by immobilized metal affinity chromatography (IMAC). The bacterial culture supernatant (1 liter) was concentrated 5-fold using a Pellicon tangential flow ultrafiltration system (Millipore, 10 kDa PGLC filter) and dialyzed overnight at 4°C against 10 liters of buffer A (50 mM Tris-HCl, pH 7.2, 0.5 M NaCl, 0.025% Tween-20, 10% glycerol, 5 mM β-mercaptoethanol, 0.02% sodium azide). The recombinant protein was allowed to bind Ni-NTA gel (25 ml; Qiagen, Chatsworth, IL) in buffer A with end-to-end mixing (1 h, 4°C), the gel was packed into a column and washed with buffer A (4 ml/min) until a stable baseline (A_{280}) was reached. Bound protein was eluted (2 ml/min) with a gradient of 0–100% buffer B (50 mM Tris-HCl, pH 7.2, 0.5 M NaCl, 1 M imidazole, 0.02% sodium azide; 50 min) followed by 100% buffer B (10 min). Elution of the L chain in the fractions (1 min each) was monitored by enzyme-linked immunoflow assay for c-*myc* [22]. Chromatography on protein L coupled to CNBr-activated Sepharose 4B was as described [26]. Size exclusion chromatography was on a Superose-12 high performance gel filtration column (Pharmacia) in 50 mM Tris-HCl, pH 7.2, 0.5 M NaCl, 0.025% Tween-20 (0.4 ml/min). The column fractions (0.4 ml) were dialyzed overnight against 50 mM Tris-HCl, pH 8.0, 0.1 M glycine, 0.025% Tween-20 using a Life Technologies multi-well apparatus and assayed for [tyr$^{10\text{-}125}$I]VIP hydrolyzing activity. In a preliminary screening experiment, the two human L chains (designated hk13 and hk14), a murine catalytic L chain (clone c23.5) and a murine non-catalytic L chain (clone c23.1) were purified from periplasmic extracts (7 ml prepared from 250

ml of the bacterial culture) on 1 ml Ni-NTA gel packed in a disposable polypropylene column (Qiagen, Chatsworth, IL) [27]. Bound L chains were eluted with 9 ml imidazole buffer (buffer B) and the eluate was assayed for the ability to hydrolyze [tyr^{10}-^{125}I]VIP. Protein purity was assessed by SDS-polyacrylamide gel electrophoresis (8–25% gradient gels, Pharmacia). Immunoblotting of the gels with anti-c-*myc* antibody was as in [22].

2.4. [tyr^{10}-^{125}I] VIP and casein-resorufin hydrolysis assays

[tyr^{10}-^{125}I]VIP prepared as in [28] was incubated with L chains at 37°C in 200 μl 50 mM Tris-HCl, 100 mM glycine, 0.025% Tween-20, pH 7.7 for 6 h and peptide hydrolysis was estimated by measuring the radioactivity soluble in 10% trichloroacetic acid. The estimates of peptide breakdown from this method are essentially identical to those from reversed-phase HPLC separation of intact and degraded fragments [3]. Initial rate data at varying substrate concentration were fitted to the Michaelis-Menten-Henri equation by non-linear regression analysis $\{v = (V_{max}[S])/(K_m + [S])\}$ (Enzfitter, Elsevier-Biosoft). The kinetic constants were also computed by a graphing routine [29], in which the concentrations of the catalyst-substrate complex [(CS)] at increasing substrate concentrations ($[S_t]$) were calculated at a series of assumed K_d values from the equation: $[CS]^2-[CS]([C_t] + [S_t] + K_d) + [C_t][S_t] = 0$. The assumed K_d value giving the best fit (by linear regression) between observed reaction velocity and [CS] represents the experimentally observed K_d for catalyst-substrate binding. The k_{cat} value was computed as the slope of the velocity versus [CS] plot. Cleavage of casein labeled with resorufin (200 μg/assay tube) was assayed according to the manufacturer's instructions (Boehringer-Mannheim) under experimental conditions identical to those used to measure the hydrolysis of VIP. Trichloroacetic acid (final concentration 5%) was added to the reaction mixture and the precipitate was removed by centrifugation (12000 × *g*, 5 min). The presence of resorufin-labeled casein breakdown products was estimated spectrophotometrically (574 nm; Ultrospec III, Pharmacia LKB). Bovine pancreatic trypsin (3080 U/mg) was from U.S. Biological Corporation, Cleveland, OH.

2.5. Immunoadsorption

L chains (200 μl; hk13, 2 nM; hk14, 0.5 nM) were treated with rabbit anti-human κ-chain antibody or control anti-human IgG (F_c specific) antibody immobilized on Sepharose 4B (0.2 ml settled gel) in 50 mM Tris-HCl, 100 mM glycine, 0.15 M sodium chloride, 0.025% Tween-20 [3]. Following incubation for 18 h at 4°C, the reaction tubes were centrifuged and unadsorbed material in the supernatant (50 μl aliquots) was assayed for [tyr^{10}-^{125}I]VIP hydrolysis activity.

2.6. Nucleotide sequencing

Nucleotide sequencing of the cDNA inserts in pCANTAB5*his*$_6$ was by the dideoxynucleotide chain termination method [30] using an Applied Biosystems DNA Sequenator (Model 373A) and primers LMB3 and fd-SEQ1 [22]. Sequence analysis was done using the GCG software package [31]. The germline V_L gene sequence database (V BASE, 1994) was provided by I.M. Tomlinson. The number of theoretically expected replacement mutations was determined [32,33] by comparison of nucleotides 1–285 of hk13 and hk14 L chains with their putative germline gene counterparts using software provided by B. Chang and P. Casali and a replacement frequency value of 0.8 as defined in [33]. When two nucleotide changes occurred in one codon, they were counted as separate events. Sequence differences in CDR3 contributed by the J region or in the framework I region encoded by the PCR back primer were ignored in this analysis.

3. Results

3.1. VIP hydrolysis by L chains

Two human L chains (designated hk13 and hk14 L chains) previously shown to display VIP binding activity [24] were studied for the ability to hydrolyze the peptide. In a preliminary experiment, the two human L chains and two control murine L chains in bacterial periplasmic extracts were fractionated by IMAC on Ni-NTA gel, a procedure that yields the recombinant proteins at purities > 95%, assessed by SDS-polyacrylamide gel electrophoresis [23,27]. The proportion of available [tyr^{10}-^{125}I]VIP (0.3 nM; 167,500 cpm) hydrolyzed by the human L chain clones hk13 (1 nM), hk14 (0.2 nM) and the murine catalytic light chain (6 nM; positive control) were 36%,

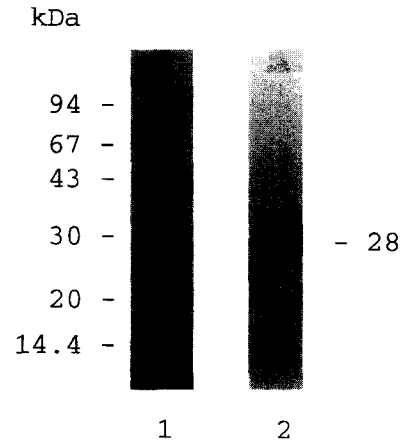

Fig. 1. SDS-polyacrylamide gel electrophoresis (8–25%) of recombinant human L chain (clone hk13). Lane 1, silver-stained L chain; Lane 2, L chain immunoblotted with anti-human κ-chain antibody.

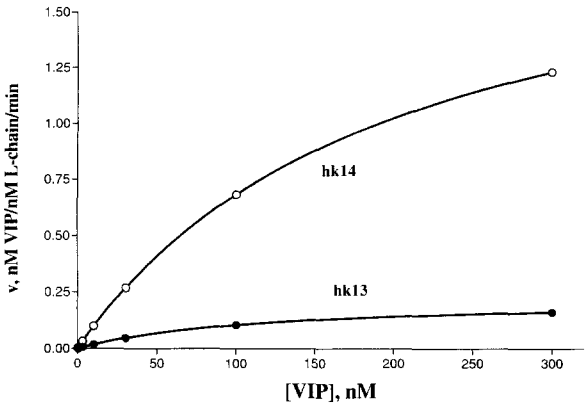

Fig. 2. Saturable VIP hydrolysis by recombinant L chain clone hk13 (0.37 nM) and hk14 (0.06 nM). VIP concentrations, 3–1000 nM mixed with a fixed concentration of [tyr^{10}-^{125}I]VIP (0.3 nM). Reaction time, 6 h. Values are means of closely agreeing duplicates.

63% and 21%. There was no detectable [tyr^{10}-^{125}I]VIP hydrolysis by the non-catalytic L chain preparation or the eluate from the periplasmic extract of bacteria in which the control vector without an L chain insert had been grown. Further characterization was performed using hk13 and hk14 L chains purified on a larger scale from the supernatants of bacterial cultures by IMAC on the Ni-NTA gel followed by chromatography on a protein L-Sepharose column. Electrophoretically homogeneous 28 kDa L chains stainable with anti-human L chain antibody (Fig. 1) and with anti-c-*myc* antibody 9E10 (not shown) were obtained by this procedure.

The hydrolysis of VIP by both L chains displayed saturation kinetics with increasing VIP concentrations (Fig. 2). The apparent K_m values were in the nanomolar range (Table 1). These K_m values are 3-orders of magnitude lower than the K_m for trypsin-catalyzed VIP hydrolysis assayed under similar conditions. Since the catalyst concentration in some assays was in the range of the low end of VIP substrate concentrations, kinetic constants were also computed by a graphing routine, which eliminates errors due to substrate depletion effects (see Section 2).

Table 1
Kinetic constants for L chain catalyzed VIP hydrolysis

Catalyst	K_m (M)	k_{cat} (min^{-1})	k_{cat}/K_m (min^{-1}M^{-1}]
hk13 L chain	1.11×10^{-7} (1.10×10^{-7})	0.22 (0.22)	2.0×10^{6}
hk14 L chain	2.02×10^{-7} (2.10×10^{-7})	2.21 (2.11)	1.1×10^{7}
Trypsin	3.80×10^{-4}	1.10×10^{3}	2.9×10^{6}

Cleavage of increasing concentrations of VIP (3–1000 nM) mixed with a constant amount of [tyr^{10}-^{125}I]VIP (0.3 nM) by hk13 L chain (0.37 nM) and hk14 L chain (0.06 nM) was estimated after incubation at 37°C for 6 h. Kinetic constants were derived from initial rates fitted to the Michaelis-Menten equation (see Fig. 2). Standard error values were < 16% of the means. Values in parentheses were computed by a graphing routine by fitting the rate data to the general equation predicting the concentrations of the catalyst-substrate at increasing concentrations of the substrate (see Section 2). Data for trypsin are from Ref. [40].

The values of k_{cat} and K_d (reported as K_m in Table 1) estimated by this method were essentially identical to the kinetic constants computed from the Michaelis-Menten equation. Since the K_m values approximate the K_d values, the observed reaction characteristics indicate comparatively high affinity binding of VIP by the L chains. The turnover number (k_{cat}) of hk14 L chain is about 200-fold greater than of a catalytic L chain elicited by immunization with VIP [25], and the kinetic efficiency (k_{cat}/K_m) of this L chain (hk14) for VIP is 3.8-fold superior than of trypsin. Turnover of the L chains, however, is slower than that of trypsin by 3–4 orders of magnitude.

Hydrolysis of casein-resorufin by hk13 L chain (0.8 μM) and hk14 L chain (0.2 μM) was undetectable by a spectrophotometric assay. In comparison, 0.2 μM trypsin displayed clearly detectable hydrolysis of this substrate (A_{574} 1.1), and the minimal concentration of trypsin at which detectable hydrolysis was observed (A_{574} 0.08) was 0.2 nM.

Immunoadsorption of hk13 and hk14 L chains with immobilized anti-κ-chain antibody resulted in near-complete removal of the VIP hydrolyzing activity. The unbound material in the supernatants recovered after incubation of hk13 and hk14 L chains with immobilized anti-κ-chain antibody hydrolyzed 2.1 ± 1.9% and 4.5 ± 1.5% of the available [tyr^{10}-^{125}I]VIP, respectively. In comparison, supernatants from hk13 and hk14 L chains incubated with the control immobilized antibody hydrolyzed 37.0 ± 3.4% and 44.3 ± 0.5% of the radiolabeled VIP, respectively. Size exclusion chromatography of hk14 L chain revealed

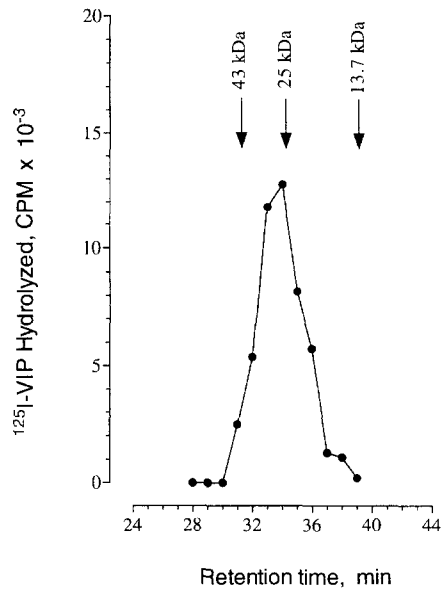

Fig. 3. Gel filtration of recombinant L chain purified by immobilized metal ion and protein L affinity chromatography (clone hk14, 1.5 μg protein). Arrows show the elution positions of marker proteins (ovalbumin, 43 kDa; chymotrypsinogen, 25 kDa; ribonuclease, 13.7 kDa). Aliquots of the column fractions (50 μl) were assayed in duplicate for [tyr^{10}-^{125}I]VIP (0.14 nM; 84300 cpm) hydrolyzing activity.

elution of the VIP hydrolyzing activity at a molecular mass corresponding to the monomer of the protein (Fig. 3). It was necessary to keep the concentration of the L chain loaded on the column to low levels (300 nM in Fig. 3) to recover the activity as shown. At a higher concentrations of the L chain (3 μM), the VIP hydrolyzing activity peak and the A_{280}-protein peak eluted in protein aggregates at the column void volume. The tendency of the L chain to form aggregates is consistent with previous observations of concentration-dependent aggregation of L chains isolated from multiple myeloma patients [34,35].

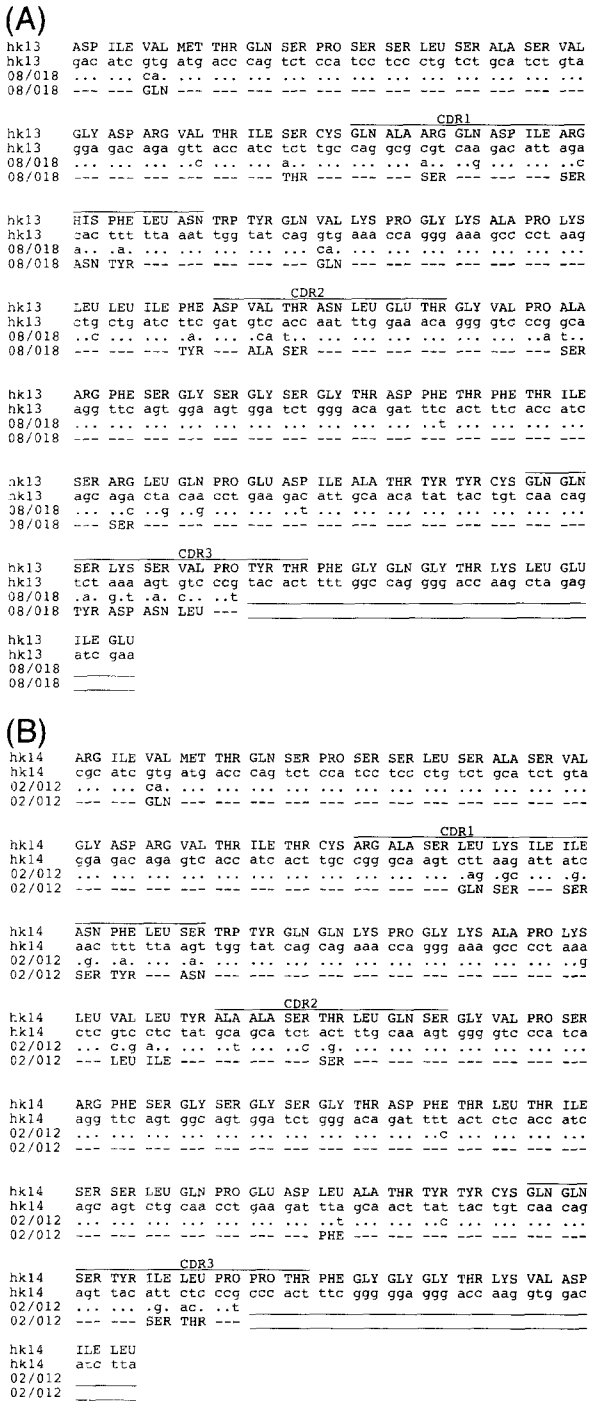

Fig. 4. Nucleotide and deduced amino acid sequences of the variable regions of L chain clones hk13 (A; GenBank accession number L43498) and hk14 (B; GenBank accession number L43499). The germline gene sequences closest in sequence to hk13 and hk14 L chains are also shown. CDRs are underlined. Dots and dashes represent conserved nucleotides and amino acids, respectively. J regions are shown as a solid line.

3.2. Sequence of L chains

Comparison of their nucleotide sequence with the Kabat database [36] suggested that both L chains belong to the κI subgroup (Fig. 4). The two L chains use different J segments; Jκ2 (hk13) and Jκ4 (hk14). According to Zachau and coworkers [37,38], the criterion for correct germline gene assignment of mature κ-chains is observation of 38 or less nucleotide nonidentities with a known germline gene. The germline V_L genes with greatest sequence identity to hk13 and hk14 L chains are O8/O18 (28 nucleotide nonidentities) and O2/O12 (23 nonidentities), respectively. Assuming that these are the germline genes of origin, both catalytic L chains appear to be extensively mutated, with the replacement mutations clustered in the CDRs. The number of replacement mutations in the CDRs is greater than in the framework regions (hk13, 11 and 6, respectively; hk14, 12 and 3, respectively). The replacement to silent mutation ratios in the CDRs and framework regions are: hk13, 11:3 and 6:7, respectively; hk14, 12:3 and 3:4, respectively. The probabilities of clustering of the replacement mutations in the L chain CDRs by a random process are: hk14, $P = 0.01$; and hk13, $P = 0.07$.

4. Discussion

The observation of sequence identities between some immunoglobulin L chains and the active site regions of serine proteases lead Erhan and Greller [39] to propose that L chains may possess proteolytic activity. This has been substantiated by observations that a polyclonal autoantibody L chain preparation [20] and the L chain of a murine antibody raised to VIP catalyze the cleavage of this polypeptide [25,40]. Contrary to the expectation that antibody catalysis is a rare phenomenon, recent observations suggest that the majority of monoclonal L chains isolated from multiple myeloma patients are capable of cleaving synthetic peptide substrates [41]. These considerations prompted us to apply phage display techniques to isolate VIP-hydrolyzing autoantibody L chains from an asthma patient. Two L chains expressed on phage particles and isolated by binding to immobilized VIP were purified to electrophoretic homogeneity. Both L chains hydrolyzed VIP. Evidence that the L chains are responsible for the observed catalytic activity consists of observations that a control non-catalytic murine L chain purified in parallel with the human L chains showed no activity, the mass of the hydrolytic activity corresponded to the monomer form

of the L chain (27 kDa), the activity was lost by immunoadsorption with specific anti-human κ-chain antibody, and the hydrolytic activity was characterized by high affinity VIP recognition.

One significant advance reported in this paper is the observation of kinetically efficient hydrolysis of VIP by the recombinant human L chains. The turnover of these L chains is in the same range as of polyclonal autoantibodies described to hydrolyze VIP [2,3] and substantially greater than of monoclonal L chains elicited by immunization with VIP [25]. Although the apparent turnover of the L chains is low compared to trypsin, their K_m for VIP is approximately three-orders of magnitude lower than of trypsin, a highly evolved catalyst. Thus, the catalytic efficiency of the L chains is comparable to that of trypsin. Trypsin hydrolyzes polypeptide substrates relatively indiscriminately. In contrast, neither L chain hydrolyzed casein, suggesting that these catalysts are specialized to recognize VIP. The K_m and K_d values of the L chain were comparable, confirming high affinity recognition of VIP by these catalysts. This is a distinguishing characteristic of natural antibody catalysis, arising from recognition of antigenic epitopes composed of multiple amino acids [42]. In comparison, conventional enzymes like trypsin display high K_m values and substrate recognition occurs primarily at amino acids at or immediately flanking the scissile bond.

The second significant observation is that the two L chains are extensively mutated compared to their putative germline gene counterparts. The replacement mutations tend to cluster in the CDRs, suggesting that the L chains are products of an antigen-driven affinity maturation process. The identity of antigens responsible for formation of autoantibodies with VIP binding and hydrolyzing activities in asthma is not known. As noted previously [19], the inciting immunogen could be endogenous VIP itself or a structurally related peptide of microbial or dietary origin. Regardless of the uncertainty about the antigenic stimulus, the development of catalytic activity in L chains appears to be compatible with the process of affinity maturation. This conclusion is supported by: (a) the presence of replacement mutations in the CDRs of the L chains described in the present study, their ability to recognize VIP with sub-micromolar K_m and their efficient peptide-bond hydrolyzing activities, and (b) frequent expression of proteolytic activity by L chains secreted by B-lymphocyte tumors in multiple myeloma patients [41]. Since the tumors in multiple myeloma patients arise at an advanced stage of B-lymphocyte differentiation [43], the catalytic activity can be assumed to survive the process of antigen-driven clonal selection.

Further analysis by site-directed mutagenesis and other techniques will be necessary to identify the three-dimensional structural composition of the catalytic sites in the L chains and the structural factors underlying their distinct properties. Seventeen of the twenty-seven amino acids constituting the CDRs of hk13 and hk14 L chains are non-identical. The putative germline genes from which the two L chains are derived are different (O8/O18 and O2/O12). Both L chains display sequence differences in their CDRs compared to a murine anti-VIP L chain with well characterized catalytic activity [25,27]. In particular, two residues identified by site-directed mutagenesis to be essential for catalysis by the murine L chain (Ser27a and His93) [27] are replaced in the human L chains by amino acids with dissimilar chemical reactivities. A Ser and an Ile residues are found at position 93 in hk13 and hk14 L chains, respectively. Neither L chain contains a position 27a residue (see Kabat et al. [36] for numbering system; position 28 in hk13 and hk14 L chains is occupied by Asp and Lys residues, respectively). Catalysis by the various L chains does not appear to be mediated, therefore, by a common catalytic site.

The availability of recombinant catalytic L chains with VIP-specific hydrolytic activity can be anticipated to facilitate the study of the functional role and utility of the catalytic antibodies. Observations of substrate-specific and kinetically efficient catalysis by autoantibodies present in autoimmune disease have raised the possibility that catalytic antibodies may mediate the pathological depletion of individual polypeptide substrates. Depletion of VIP has been observed in the airways obtained at autopsy from patients with fatal asthma [44], although analysis of tissues from patients with less severe asthma does not reveal decreased VIP levels [45]. Autoantibodies to VIP are also found in lupus. In view of the potent anti-inflammatory effects of VIP [16,46,47], it can be hypothesized that the autoantibodies contribute in the generalized increase in inflammatory reactions seen in lupus. The L chains described in the present study offer a means to achieve depletion of VIP and study the functional consequences of this event, for example, in passively immunized experimental animals. The anti-VIP L chains also offer the first opportunity to examine the therapeutic utility of catalytic antibodies. Various tumor cells are known to synthesize VIP and utilize this peptide as a growth factor via an autocrine mechanism [17,18]. Study of the growth inhibitory effect of catalytic antibodies to VIP in these types of tumors is warranted.

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