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Selection of Functional Human Immunoglobulin Light Chains from a Phage-Display Library

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ABSTRACT

Human κ-light chains (L chains) were amplified by the reverse transcriptase-polymerase chain reaction (PCR) and cloned into a phagemid vector. Phage particles displaying L chains were fractionated on immobilized vasoactive intestinal peptide (VIP). The resultant phage preparation displayed saturable binding of (tyr^{10}-^{125}I)VIP. One of the L-chain clones (hk13) was deduced to be related to subgroup I of κ-light chains based on its nucleotide sequence. The VIP binding activity of the soluble and phage-displayed form of this L chain was confirmed by radioimmunoassay and ELISA, respectively. These observations demonstrate the potential of selecting antigen-specific L chains from phage-display libraries.

Index Entries: VIP; light chains; phage-display libraries.

INTRODUCTION

Autoantibodies to vasoactive intestinal peptide (VIP) found in patients with asthma are capable of efficient cleavage of this peptide (*1,2*). The L chains purified from the IgG of a human subject positive for VIP binding and hydrolyzing autoantibodies have been observed to hydrolyze VIP with specific activity 32-fold greater than that of Fab (*3*). The L chain of a monoclonal antibody binds VIP with affinity only fivefold lower than that of the parent antibody (*4*). These findings raise the possibility that the heavy (H chains) and L chains may provide distinct contributions in the binding and hydrolysis of VIP by antibodies. Moreover, free L chains are

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known to play a regulatory role in the normal immune response, and their interaction with the antigen may contribute in the pathophysiology of lymphocyte tumors associated with accumulation of L chains in blood and tissues (*5,6*).

The availability of recombinant L chains with catalytic activity will significantly facilitate further examination of their antigen binding and hydrolyzing activity. Here, we describe the selection of recombinant L chains with VIP binding activity from a human phage-display library.

METHODS

cDNA Preparation

A κ-L-chain cDNA library was prepared from the peripheral blood lymphocytes of a patient with asthma. Lymphocytes (3×10^8) were obtained by leukopheresis and density-gradient separation on Lymphoprep (Nyegaard & Co., Norway). Total RNA was prepared by the method of Chomczynski and Sacchi (*7*). cDNA was prepared using reverse transcriptase and a 3′ primer corresponding to the constant region of κ-L-chains, 5′ CCA TCC TGC GGC CGC ACA CTC TCC CCT GTT GAA GCT CTT-3′. This 3′ primer and family-specific degenerate 5′ primers were used to amplify the κ-L-chain cDNA by PCR (*8,9*). The 5′ and 3′ primers contained *Sfi*I and *Not*I restriction sites, respectively, to permit cloning of the PCR product.

Phage Display and Secretion of L Chains

PCR products obtained from the amplification of κ-L-chain cDNA were cloned into the phagemid vector pCANTABShis$_6$ via *Sfi*I and *Not*I restrictions sites (*10*). This expression system permits display of cloned cDNA products on the surface of phage particles fused to the N terminus of the geneIII coat protein. *E. coli* TG1 cells were transformed with the cDNA by electroporation, and following selection of ampicillin-resistant cells, phagemid particles were was rescued usiing VCSM13 helper phage (Stratagene) as described (*9,10*). The phage partaicles were concentrated by two rounds of polyethylene glycol (PEG) precipitation and subjected to affinity chromatography. Approximately 10^{13} plaque-forming units of phage/mL were incubated for 8 h with 1 mL of 0.52 mg VIP immobilized on CNBr-Sepharose (*11*) in phosphate-buffered saline (PBS; 1.9 m*M* NaH_2PO_4, 8.1 m*M* Na_2HPO_4, 150 m*M* NaCl, pH 7.4) containing 2% w/v nonfat milk with end-to-end mixing. The gel was packed in a column and washed with buffers of increasing pH (150 mL PBS, 20 mL 50 m*M* Tris-

HCl, pH 7.5; 20 mL 50 m*M* Tris-HCl, pH 8.5; 20 mL 50 m*M* Tris-HCl, pH 9.5; 20 mL 50 m*M* $NaHCO_3$, 0.5*M* NaCl, pH 9.6). Phage particles bound to VIP were eluted with 0.1*M* triethylamine, pH 5.5, neutralized, and used to infect logarithmically growing *E. coli* TG1. Phagemid particles were rescued again by superinfection with helper phage. The concentration of the phage particles concentrated by PEG precipitation was determined photometrically (269 nm, absorption coefficient, 3.84 $mg^{-1}cm^{-1}$ [*12*]). To screen for individual VIP binding clones (*see below* for binding assay method), phage particles eluted from the VIP-Sepharose column (pH 5.5) were grown in *E. coli* (strain HB2151) in agar. The amber codon between the L-chain insert and geneIII allows for expression of L chains as soluble proteins in this bacterial strain. Individual clones were picked, grown in 96-well microtiter plates, and induced with isopropyl-β-D-thiogalactopyranoside (IPTG) for production of soluble L chains. To prepare periplasmic extracts, cells were pelleted (4000 x *g*, 10 min), and pellets were resuspended in lysate buffer (10 m*M* sodium phosphate buffer, 1*M* NaCl, 1 m*M* EDTA, pH 7.5). Following 30 min incubation on ice, lysates were centrifuged at 6000 x *g*, and phenylmethylsulfonyl fluoride (5 μg/mL) was added to the supernatant. The extracts were tested for expression of L chains by immunoblotting with anti-*c-myc* 9E10 monoclonal antibody (*13*) and for VIP binding as described below. Phagemid DNA from VIP binding clones was sequenced by the dideoxynucleotide chain termination method using an Applied Biosystems DNA Sequenator (Model 373A), primers LMB3 (upstream from the leader sequence), and fd-SEQ1 (5′-end of geneIII) (*9*).

VIP Binding

Phage preparations (final concentration 480 μg protein/mL) were incubated with a constant amount of (tyr^{10}-^{125}I)VIP (38 p*M*) and increasing concentrations of unlabeled VIP for 8 h at 4°C in radioimmunoassay buffer (*14*). Bound and unbound radioactivity was separated by PEG (20% w/v) precipitation, and the radioactivity in the pellet was counted. An ELISA was used to confirm VIP binding by the L chains displayed on the phage surface. VIP conjugated to keyhole limpet hemocyanin (*15*) was coated on 96-well polyvinylchloride plates (Costar) (10 μg/mL in a carbonate/bicarbonate buffer, pH 9.6; 50 μL/well; 8 h). Nonspecific binding sites were blocked for 2 h with 5% bovine serum albumin (Sigma) in PBS buffer containing 0.05% Tween 20. PEG-precipitated phage particles were preincubated with the 5% BSA buffer (1 h) and incubated in VIP-coated wells (2 h, room temperature). Following removal of unbound particles by washing, a peroxidase-labeled anti-M13 antiserum (Pharmacia recombinant antibody detection module kit) was used to detect phage particles bound to the solid phase as described by the supplier.

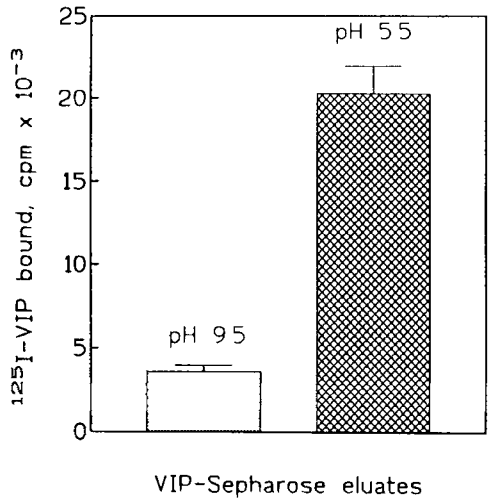

Fig. 1. Binding of ^{125}I-VIP by a selected human κ-L-chain library expressed on the surface of phage particles. The binding of particles (480 μg/mL) eluted from the VIP-Sepharose affinity column with pH 9.5 or 5.5 buffers is shown. Total available radioactivity was approx 35,000 cpm in an assay volume of 150 μL.

RESULTS

An immunoglobulin κ-L-chain cDNA library was prepared from the unstimulated lymphocytes of a patient with asthma. The cDNA was cloned into the phagemid vector pCANTABhis$_6$ and phage particles displaying the L-chain library were fractionated by affinity chromatography on VIP-Sepharose. The particles eluted from the affinity column at pHs 9.5 and 5.5 were tested for VIP binding (Fig. 1). The greatest level of binding was observed with particles eluted at pH 5.5. Low-level binding by particles eluted at pH 9.5 was nonsaturable, indicated by observations that unlabeled VIP at concentrations up to 100 μM did not inhibit the binding (Fig. 2). In comparison, the particles recovered at pH 5.5 displayed progressively decreasing (tyr^{10}-^{125}I)VIP binding at increasing concentrations of unlabeled peptide (50% inhibition at 1.5 μM VIP).

The phage particles eluted at pH 5.5 were used to infect *E. coli* HB2151 to produce soluble L chains. Individual bacterial clones picked from agar plates were grown in 96-well plates and induced with IPTG. The supernatants were tested for VIP binding by radioimmunoassay. Fifty-six percent of the clones displayed (tyr^{10}-^{125}I)VIP binding displaceable by 100 μM VIP. The levels of saturable binding by L chains in the periplasmic extract of one of the clones (hk13) are shown in Table 1. A control periplasmic extract (from *E. coli* HB2151 transformed with pCANTAB5his$_6$ without an L-chain insert) did not display binding of VIP. The periplasmic extract from clone hk13 was subjected to SDS-polyacrylamide gel

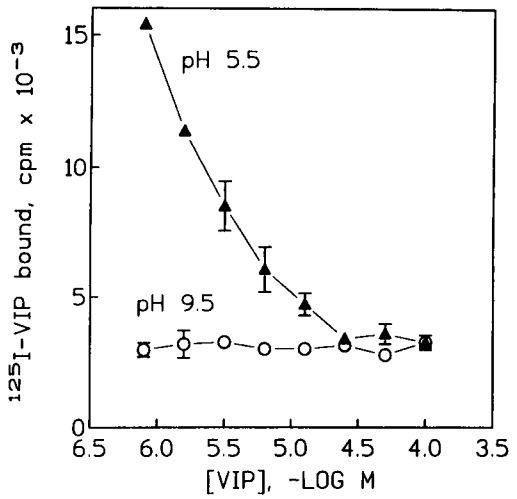

Fig. 2. Saturable ^{125}I-VIP binding by phage particles displaying κ-L-chains. The particles were recovered from the VIP-Sepharose column in pH 9.5 or 5.5 buffers. Assay conditions were as in Fig. 1.

Table 1
^{125}I-VIP Binding by Soluble hk13 L Chain

Extract[1]	^{125}I-VIP saturable binding, CPM ± SD[2]
pCANTAB5his$_6$	127 ± 543
hk13	5676 ± 260

[1]Periplasmic extracts of HB2151 cells transformed with phagemid; 10 μg protein/mL.

[2]CPM bound in the absence of unlabeled VIP; CPM bound in 4 μM VIP; bound ^{125}I-VIP was precipitated with anti-c-*myc* monoclonal antibody 9E10 1:800 dilution of ascites (3 h, 4°C), addition of PEG to 3%, and centrifugation.

electrophoresis, and the gels were immunoblotted with anti-c-*myc* monoclonal antibody 9E10. A 30-kDa anti-c-*myc* stainable band was observed, confirming expression of soluble recombinant L chains (Fig.3). An ELISA was employed to demonstrate VIP binding by the displayed L chain on the phage surface. The phage particles from hk13 were bound by immobilized VIP in this ELISA, determined by staining with a peroxidase-conjugated antiphage antibody (OD_{492} 0.62 [hk13], 0.17 [vector without insert; negative control]). Based on its deduced amino acid sequence (Fig. 4), the hk13 L chain belongs to the κ-chain subgroup I.

1 2

Fig. 3. Anti-c-*myc* (9E10) stained immunoblot of an SDS-electrophoresis gel of hk13 (periplasmic extract). 1, hk13; 2, a positive control single chain F_v clone (courtesy R. Smith).

D I V M T Q S P S S L S A S V G D R V T I S C Q A R Q N I R H F L N W Y Q V K P
20
G K A P K L L I I F D V T N L E T G V P A R F S G S G S G T D F T F T I S R L Q
60
Q P E D I A T Y Y C Q Q S K S V P Y T F G Q G T K L E I E R T
100

Fig. 4. Deduced amino acid sequence of the variable region of hk13 L chain. CDRs are underlined.

DISCUSSION

Free immunoglobulin L chains apparently retain some VIP binding activity. More significantly, they hydrolyze the antigen more efficiently than the intact IgG molecule (*3,4*).

We are exploring the utility of a phage-display system in isolating human L chains with VIP binding and hydrolyzing activities. The results of the present study suggest that selection of immunoglobulin subunits with detectable VIP binding activity is possible, even from a restricted L-chain library prepared from an unimmunized asthma patient. The buffer used in the present study to determine (tyr^{10}-^{125}I)VIP binding by the recombinant L chains contained a large excess of albumin (0.1% w/v; 190-fold excess over radiolabeled VIP), suggesting that the binding activity is peptide-selective. Moreover, we identified several recombinants without binding activity. Preliminary studies reported here suggest that the binding affinity of the L chains is in the micromolar range. Since VIP is an amphiphilic peptide known to bind several proteins and solid surfaces weakly, careful study of its interactions with soluble and phage-displayed L chains will be needed to define the specificity characteristics of this system.

Previous studies do not show unambiguously whether the monomer, dimer, or both forms of pure H-chain (*16*) and L-chain (*4*) subunits are capable of antigen binding. Since display on the phage surface is likely to be in the form of individual chains, the results of the present study suggest that monomeric L chains can recognize VIP. In principle, phage-displayed L chains trapped on the VIP-Sepharose column may be catalytic,

since the affinity of the catalytic L chains is sufficient (4) to permit retention on affinity columns. Nevertheless, it remains that the best binders may not be the best catalysts, a limitation that can potentially be overcome by development of technologies permitting direct selection of catalysts, e.g., affinity separations based on binding by suicide inhibitors of catalytic antibodies (*17*).

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DISCUSSION

Sonia Tyutyulkova

Green: You mentioned that there are theoretical reasons for thinking that the L chain would be more catalytically active than the intact antibody. Can you elaborate?

Tyutyulkova: One reason for that expectation is that there is homology between serine proteases and CDR1 of some L chains. Another reason is that catalysis by antibodies that bind the substrate tightly may be more difficult than by the subunits. L chains, for example, appear to bind the substrate weakly.

Zouali: Which family of light chains? Do you have the complete sequence of the L chain?

Tyutyulkova: Yes. The L chain belongs to Subgroup I according to Kabat and Wu.

Gabibov: Do you know if the L chain is catalytic?

Tyutyulkova: No. We will need to purify the L chains to homogeneity in order to test for catalysis.

Gabibov: Is the sequence of the L chain homologous to known proteases?

Tyutyulkova: We do not see an unambiguous homology between the L chain and known enzymes.

Gabibov: What is the K_d for the binding?

Tyutyulkova: Careful affinity measurements remain to be done. From the competition curves, IC_{50} values are in the micromolar range.

McCafferty: Do you know anything about crossreactivity of the L chain with other proteins.

Tyutyulkova: We have not performed detailed antigen-specificity experiments.

Paul: BSA was present in excess in the binding assay, so the L chains are likely to have some specificity for VIP.

McCafferty: Do you know if the L chain binds as a monomer or dimer?

Tyutyulkova: In soluble form, the L chains might dimerize at high concentration. On the phage surface, they are likely to be displayed as monomers. We observe VIP binding by both forms of L chains.

McCafferty: Yes. In the pCANTAB5 phagemid system, the majority of the particles are likely be be bald. Perhaps 1 in 10 or 1 in 20 particles may express a single copy of the fusion protein.