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Hepatitis B virus-neutralizing anti-pre-S1 human antibody fragments from large naïve antibody phage library

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Abstract

We report the construction of a large nonimmunized human phage antibody library in single-chain variable region fragment (scFv) format, which allowed the selection of antibodies that neutralize hepatitis B virus (HBV) in vitro. We generated 1.1×10^{10} independent scFv clones using the cDNA of functional variable (V) gene segments of heavy and light chains purified from the peripheral blood mononuclear cells of 50 nonimmunized human donors. Using BIAcore, we selected two clones that recognized pre-S1 and neutralized pre-S1 and HBV binding to Chang liver cells. Clone G10 had the highest affinity ($K_D = 1.69 \times 10^{-7}$ M), which was higher than that of clone 1E4 that was generated previously from a heavy chain-shuffled immune library. The off-rates of clones were within 10^{-3} s^{-1} as determined by BIAcore and were comparable to those of antibodies derived from a normal secondary immune response. In the inhibition assays of pre-S1 and virus binding to Chang liver cells using flow cytometry and the polymerase chain reaction, G10 had better neutralizing activity than 1E4. The new phage library may be a valuable source of antibodies with reasonable affinities to different targets, and the anti-pre-S1 G10 may be a good candidate for immunoprophylaxis against HBV infection.

Keywords: Naïve antibody library; Single-chain variable region fragments; Pre-S1 protein; BIAcore

1. Introduction

Expressing cDNAs or peptides on filamentous phages has been a powerful tool for the identification of functional peptides or proteins with pharmaceutical applications (Clackson and Wells, 1994; Winter et al., 1994; Bradbury and Marks, 2004; Ladner et al., 2004). Among functional proteins, antibodies are of particular interest owing to their ability to recognize a variety of targets with high specificity and affinity. More specifically, the use of partial or complete human antibodies, which elicit no (or a minimal) immune response when administered to patients, is yielding a growing list of Federal Drug Administration-approved protein-based drugs (Brekke and Loset, 2003). Phage display technology enables the generation of large repertoires of human antibodies (McCafferty et al., 1990; Sblattero and Bradbury, 2000; Huie et al., 2001), while biopanning permits the selection of individual

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antibodies with a desired specificity. Using the phage display system, various formats of antibody fragments can be displayed on the surface of filamentous phages that contain the antibody genes (Smith, 1985; Clackson et al., 1991; Fernandez, 2004). Antibody fragments such as single-chain variable region fragment (scFv) molecules have been developed for potential clinical applications (Fitch et al., 1999; Mahaffey et al., 2003). ScFvs are the smallest type of antibody fragment and are composed of a light and a heavy chain variable region (VL and VH, respectively) joined by a short peptide spacer.

The natural primary antibody repertoire within B cells contains a large array of antibodies that recognize a variety of antigens; this array can be cloned as a 'naïve' repertoire of rearranged genes by incorporating the V genes from IgM mRNA of B cells of nonimmunized human donors, or from total RNAs of peripheral blood lymphocytes (Holliger and Hoogenboom, 1998), bone marrow, or tonsils (Vaughan et al., 1996), and from similar animal sources (Gram et al., 1992). Even though immune libraries from immunized humans or patients with infectious diseases would be more likely to have high possibility to select antibodies against target agents, they are not effective at generating antibodies against a large panel of antigens including self, non-immunogenic, and relatively toxic antigens which usually make it difficult to mount antibody responses in vivo (Griffiths et al., 1993). To avoid this problem associated with immune library, naïve antibody libraries were generated from the nonimmunized donors, which has proven to contain universal source of binders. However, a major drawback of the naïve libraries is that antibodies selected from the library usually show low or moderate affinities (10^4–$10^6\,M^{-1}$) (Nissim et al., 1994). Over the last few years, more efficient techniques have been developed to create larger libraries of antibody fragments using sophisticated in vivo recombination methods (Griffiths et al., 1993) or brute-force cloning procedures (Sheets et al., 1998) in an effort to improve the affinity of recombinant antibodies in naïve library. Recently, many reports revealed that very large naïve or semi-synthetic antibody libraries offered the possibility to select high affinity antibodies with any desired specificity without the need for immunization (Lee et al., 2004). Such larger libraries have yielded a greater number of human antibodies per antigen, and these antibodies have a much higher affinity (up to subnanomolar) on average than smaller libraries (de Haard et al., 1999; Lee et al., 2004). The in vitro selection process involves the sequential enrichment of specific binding phages from a large excess of non-binding clones through reamplification of specific binders, a process that generally reduces the library diversity and requires relatively long selection time. However, surface plasmon resonance-based system such as BIAcore can circumvent this problem associated with in vitro selection by allowing more rapid selection and identification of clones with adequate affinities on the basis of binding kinetics (Malmborg et al., 1996).

Many reports have shown that the pre-S1 of hepatitis B virus (HBV) envelope is responsible for attaching to and infecting hepatocytes (Neurath et al., 1985; Le Seyec et al., 1999), a process that can be inhibited by anti-pre-S1 antibodies (Neurath et al., 1989). Therefore, anti-pre-S1 human monoclonal antibodies may be used as therapeutic reagents to prevent HBV infection in humans.

To our knowledge this is the first human anti-pre-S1 scFv antibody selected from a large naïve human antibody library, which shows neutralizing activity against pre-S1 and HBV virion binding to Chang liver cells. In this study, we described the use of a BIAcore flow system to select anti-pre-S1 human scFvs from the library and of a flow cytometry to analyze the pre-S1 binding to cells. The neutralizing scFvs may be good candidates for immunoprophylaxis against HBV infection.

2. Materials and methods

2.1. Construction of naive human scFv phage library and preparation of phages

Fig. 1 is a flow diagram illustrating the construction of a naive human scFv phage library. As a source of lymphoid tissue, we used peripheral blood mononuclear cells (PBMC) from 50 healthy donors. Mononuclear cells were isolated on a Ficoll-Pacque gradient. Total RNA was extracted from each sample using Trizol (GibcoBRL/Life Technologies, Gaithersburg, MD). First-strand cDNA was generated using Superscript II reverse transcriptase (RT; Invitrogen, Carlsbad, CA). Cloning of light- and heavy-chain genes was done by the polymerase chain reaction (PCR) using the primers described by Barbas et al. (2001). The variable regions of light (VL)- and heavy (VH)-chain genes were amplified separately from each cDNA and were recombined by a second round of PCR. A pool of gene fusions that encoded scFv of the VL–spacer–VH sequence was assembled (spacer: GGSSRSSSSGGGGSGGGG). Following overlap PCR and gel purification, the amplified product was digested with *Sfi*I before being purified and ligated into the phagemid vector pComb3H. *Escherichia coli* ER2537 cells were electroporated (2.5 kV, 25 μF, 200 Ω) with the ligation mixtures using Gene Pulser II (Bio-Rad Laboratories, Munich, Germany). Library phages were harvested from the culture supernatant of recombinant *E. coli* and precipitated with 20% PEG/2.5 M NaCl as described previously (Park et al., 2000). The phage pellet was reconstituted in 2 ml of 1% (w/v) bovine serum albumin (BSA) in Tris-buffered saline (TBS; 50 mM Tris–HCl, 150 mM NaCl, pH7.5) before being filtered through a 0.45-μm filter. The cloning efficacy and diversity of the library were determined by PCR screening using ompseq (5′-AAGACAGCTATCGCGATTGCAG-3′) and gback (5′-GCCCCCTTATTAGCGTTTGCCATC-3′) primers. The amplified product was digested with a frequent-cutting enzyme, *Bst*NI (NEB), and was analyzed on agarose gels.

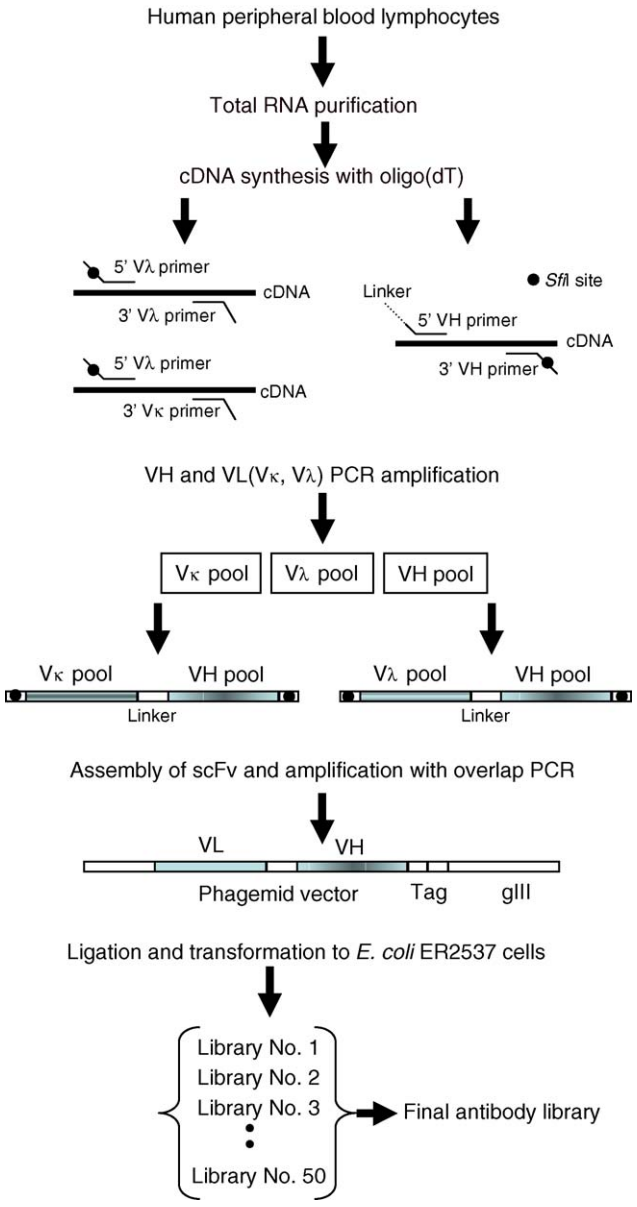

Fig. 1. Flow diagram illustrating the construction of the single-chain variable region fragment (scFv) phage library.

2.2. Surface plasmon resonance

A BIAcore 2000 instrument (Biacore AB, Uppsala, Sweden) was used to (a) select phages from the library, (b) screen for high binding affinity, and (c) analyze the kinetic properties of scFvs. Pre-S1 was immobilized on a 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride/*N*-hydroxysuccinimide-activated CM5 sensor chip by injecting 20 μg/ml pre-S1 in 10 mM sodium acetate (pH 5.0) to obtain 300–500 resonance units for phage selection and screening or 150–200 resonance units for the kinetic assay. For phage selection, 100 μl of the phage library (1×10^{12} colony-forming units/ml) were injected at 1 μl/min before the sensor chip was washed with HBS-EP buffer (0.01 M HEPES pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.005% Surfactant P20; Biacore AB) by flowing the buffer continuously for 6 h. The phages eluted with regeneration buffer (50 mM NaOH, 1 M NaCl) were small-scale amplified in 96 deep-well plates (Nunc, Rockilde, Denmark) and were then analyzed with regard to the binding kinetics with the pre-S1-immobilized sensor chip at 2 μl/min. The flow cell was regenerated between samples using sequential injections of 0.5× regeneration buffer without significant changes in the sensorgram baseline after analysis of more than 100 samples. For the kinetic assay of soluble scFvs, measurements were carried out at a flow rate of 30 μl/min. Because varying the flow rate within the range of 25–100 μl/min did not alter the association rates, we concluded that mass transfer effects did not compromise the analyte. The rate constants of association (k_{on}) and dissociation (k_{off}) were obtained at five different scFv concentrations (range: 1–100 μg/ml). All assays were repeated at least three times. The dissociation constant (K_D) was calculated from the ratio of the rate constants of association and disassociation (k_{on}/k_{off}). Sensorgrams were analyzed with BIAevaluation 2.1 software. Residual scFv was removed with 50 mM NaOH after each measurement.

2.3. DNA sequencing

The DNA that encoded the scFv was sequenced using a BigDye Terminator Ready Reaction Kit (Applied Biosystem, Foster, CA). Plasmids of selected clones were prepared using QIAprep Spin Miniprep Kit (Qiagen, GmbH, Germany). HRML-F (5′-GGTGGTTCCTCTAGATCTTCC-3′) and ompseq primers (Barbas et al., 2001) were used for sequencing.

2.4. Expression and purification of soluble scFvs

The induction of scFv expression was performed as described previously (Park et al., 2000). Briefly, the Top10F' strain of *E. coli* was infected with selected phages before cells were grown to the logarithmic phase and induced with 0.1 mM isopropyl-β-D-thiogalactopyranoside overnight to produce soluble scFvs. Expressed scFv was purified by immobilized metal affinity chromatography with a Ni^{2+}-charged HP chelating column using the AKTA explore FPLC system as recommended by the manufacturer (Amersham-Pharmacia Biotech, Uppsala, Sweden). To separate monomeric, dimeric, and aggregated scFv, samples were fractionated on a Superdex 75 column. The purity of the final preparation was evaluated by assaying an aliquot by sodium dodecylsulphate-polyacrylamide gel electrophoresis. Protein bands were detected by Coomassie staining. The protein concentration was determined with the Bradford reagent according to the manufacturer's instructions (Bio-Rad).

2.5. Cell lines and virus

A human hepatoma cell line, Chang liver cells (American Type Culture Collection, Rockville, MD), were grown

in RPMI 1640 with 10% fetal calf serum. The HepG2 2.2.15 cell line, which produces mature HBV virions, was a gift from Dr. ACS (Sells et al., 1987). The supernatant from HepG2 2.2.15 cells cultured in H medium (75% minimal essential medium and 25% medium 199 supplemented with 5 mg/l insulin, 4.5 mg/l penicillin, 50 mg/l streptomycin, 3.5×10^{-7} M hydrocortisone hemisuccinate, 2 mM L-glutamine, and 10% fetal calf serum) was collected every 24 h and stored at −70 °C until use. Virus titers of culture supernatants were measured using the COBAS Amplicor HBV monitor test V2.0 (Roche, Indianapolis, IN) according to the manufacturer's instructions.

2.6. Inhibition of binding of pre-S1 using scFv

Cells (1×10^5) were incubated with 5 μg/ml of biotinylated pre-S1 (pre-S1-biotin) and biotinylated pre-S2 (pre-S2-biotin) for 1 h at room temperature. Cells were stained with phycoerythrin (PE)-conjugated streptavidin (Jackson ImmunoResearch Laboratories, West Grove, PA) for 30 min at 4 °C. Pre-S1 and pre-S2 proteins were produced and purified from *E. coli*. For the inhibition of binding (IOB) assay, 5 μg/ml of pre-S1-biotin was preincubated with various concentrations of scFv for 1 h at 4 °C and the reaction mixtures were added subsequently to the cells. After incubation for 1 h, cells were developed with PE-conjugated streptavidin as described above. Cells were washed twice with fluorescence-activated cell sorting (FACS) buffer (1% BSA, 0.1% NaN_3 in phosphate-buffered saline) and were analyzed using a FACSort flow cytometer (BD Biosciences, San Diego, CA).

2.7. Inhibition of binding of HBV virions using scFv

HBV virions (10^4 copies) from the HepG2 2.2.15 cell line were preincubated with various concentrations of scFv for 1 h at room temperature. Cells were then washed five times with phosphate-buffered saline. All cells were boiled for 20 min 20 μl of distilled water for the extraction of DNA. Relative amounts of viral particles bound to the cells were measured by PCR using PS1 (5′-GGGTCACCATATTCTTGG-3′) and PS2 (5′-GTCCTAGGAATCCTGATG-3′) primers. The thermal cycling protocol was 30 cycles at 94 °C for 30 s, 56 °C for 20 s, and 72 °C for 60 s.

3. Results

From the PBMCs of 50 healthy volunteers, total RNA was extracted and the cDNA fragments that encoded VL and VH genes were amplified individually by RT-PCR (Fig. 2A). VL and VH genes were converted to VL–spacer–VH-type scFv genes by overlap PCR (Fig. 2B), and these scFvs were cloned into the phagemid vector, pComb3H, which allowed the fusion of the VH fragment to gene III protein of phage minor coat protein. Each scFv library was cloned separately and then these were merged into a single library. The final nonimmune human scFv library contained 1.1×10^{10} individual clones. To ascertain the quality of the library, DNA segments that encoded scFv genes from 16 randomly selected clones were amplified and digested with *Bst*NI. The fingerprint patterns were diverse enough, which was suggestive of substantial diversity in the nonimmune human scFv library (Fig. 2C).

BIAcore is a surface plasmon resonance-based system that is used to analyze biospecific interactions in real time. It can discriminate and select phage antibodies on the basis of kinetics and rates of association and dissociation (Malmborg et al., 1996). We used BIAcore to select and screen pre-S1 binders. Phage antibodies (1×10^{11} colony forming units) were reacted with pre-S1 that had been immobilized on CM5 sensor chips. Following regeneration, we recovered 67 clones. After screening these clones, we obtained two phage antibodies that exhibited a lower off-rate than the remaining clones (Table 1), and analyzed the kinetic parameters of these clones using their soluble scFvs. The affinities of the two selected antibodies were 1.69×10^{-7} and 1.12×10^{-6} M and the off-rates were 2.05 and 5.56×10^{-3} s^{-1}. The nucleotide

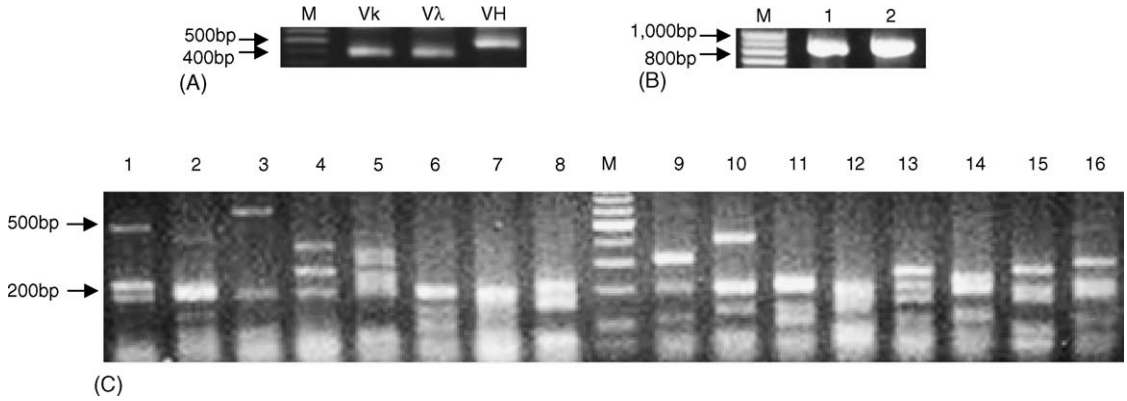

Fig. 2. Construction of nonimmune (naïve) human scFv library. (A) The variable regions of lambda-, kappa-, and heavy-chain (and V_κ, V_λ, and VH, respectively) sequences amplified from human peripheral blood mononuclear cells. (B) Preparation of scFv genes. Lane 1, V_κ–VH; lane 2, V_λ–VH. (C) Nonimmune scFv that encoded inserts of 16 phage clones were amplified by the polymerase chain reaction (PCR) using ompseq and gback primers. The amplified products were digested with the *Bst*NI at 60 °C for 2 h. The restriction patterns of samples were analyzed on agarose gels.

Table 1
Affinities and binding kinetics of anti-pre-S1 single-chain variable region fragments (scFvs)

ScFv clone	K_D (10^{-7} M)	k_{on} (10^4 s^{-1} M^{-1})	k_{off} (10^{-3} s^{-1})
G10	1.69	2.59 ± 0.12	2.05 ± 0.36
H9	11.2	0.52 ± 0.24	5.56 ± 0.19
1E4	2.58	2.05 ± 0.28	5.24 ± 0.28

K_D, dissociation constant; k_{on}, rate constant of association; k_{off}, rate constant of dissociation. We measured k_{on} and k_{off} by surface plasmon resonance in a BIAcore to calculate K_D.

sequences of the two clones were analyzed with IgBlast from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/igblast/). The VH genes are derived from the VH3 and VH5 families; the VL genes belong to the V_κ and V_λ families (Table 2). The amino acid mutation frequencies of the selected antibodies were 9.8% for the VH segments (17 amino acid mutations of 174) and 9.6% for the VL segments (21 of 118) based on the germline sequences (data not shown).

To evaluate the biological effects of anti-pre-S1 scFvs, we first examined the binding of pre-S1 to Chang liver cells by incubating cells with pre-S1 or pre-S2-biotin, and then analyzed the binding by flow cytometry. As shown in Fig. 3A, pre-S1 bound to Chang liver cells, whereas pre-S2 did not. Next, to test the neutralizing activity of anti-pre-S1 scFvs, 5 μg/ml of pre-S1-biotin was preincubated with serially diluted scFv fragments, and these mixtures were reacted with cells. The results showed that pre-S1 binding was inversely correlated with amount of scFv in dose-dependent

Table 2
Variable (V) gene segments and CDR3 sequences of anti-pre-S1 single-chain variable region fragments (scFvs)

ScFv clone	Family	Germline gene	CDR3	Amino acid changes from germline[a]
(A) Heavy chains				
G10	VH3	3–74	ETWYRCDY	13
H9	VH5	5–51	GPYYDSWSGRPLSFWFDP	14
(B) Light chains				
G10	V_κ 1	L12	QQYETYPYT	16
H9	V_λ 1	V1–16	ATWDDSLKAVV	5

VL, light-chain variable region; VH, heavy-chain variable region. Nucleotide sequences have been submitted to the National Center for Biotechnology Information Gene Bank with the following accession numbers: G10, AY879804 (VH), AY879803 (VL); H9, AY879801 (VH), AY879802 (VL).
[a] Amino acid differences in the V-gene segment, excluding the FR-1 region encoded by the primers used for cloning, and CDR3.

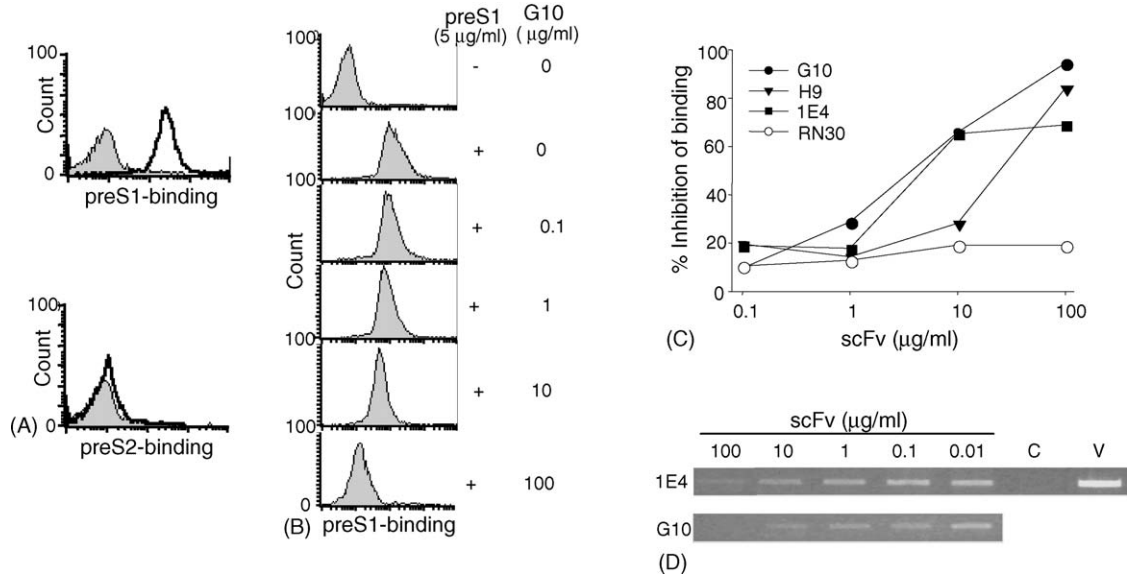

Fig. 3. Inhibition of binding (IOB) of pre-S1 and HBV virions. (A) Biotinylated pre-S1 and pre-S2 protein cell-binding assay. Chang liver cells were incubated with 5 (g/ml pre-S1 or pre-S2-biotin at room temperature for 1 h. Cells were stained with PE-conjugated streptavidin and were analyzed by flow cytometry (open histograms). The control (nonspecific fluorescence, filled histograms) was measured by adding phycoerythrin (PE)-conjugated streptavidin to cells in the absence of pre-S1-biotin. (B) Histograms of inhibition of pre-S1 binding to Chang liver cells by the anti-pre-S1 scFv, G10. Pre-S1-biotin (5 μg/ml) was preincubated with serially diluted G10 scFv fragments at 4 °C for 1 h. Cells were incubated with reaction mixtures and stained with PE-conjugated streptavidin. (C) Detailed IOB of pre-S1 using anti-pre-S1 scFvs or control scFv (RN30). The percent inhibition was calculated as [(MFI of pre-S1 alone − MFI of pre-S1 with scFv)/(MFI of pre-S1 alone − MFI of blank)] × 100. Mean values of duplicate measurements are shown. (D) IOB of HBV virions. Virions (10^4 copies) from HepG2 2.2.15 cells were preincubated with various concentrations of scFv (0.01–100 (g/ml) fragments for 1 h at room temperature. Reaction mixtures were incubated with Chang liver cells (1×10^5) for 1 h at room temperature. DNA from HBV bound to cellswas extracted, amplified with PCR, and were analyzed on agarose gels. Lane C, uninfected cells; lane V, cells exposed to HBV alone.

mode, suggesting that G10 specifically blocked pre-S1 binding to Chang liver cells (Fig. 3B). All anti-pre-S1 scFvs had inhibitory activity against pre-S1 binding, and among them G10 showed the greatest inhibitory activity ($IC_{50} \approx 4$ μg/ml) (Fig. 3C). RN30, a human scFv that was raised in our laboratory against RNase H of HBV, was used as a negative control and had no inhibitory effect (Fig. 3C). All scFv tested did not bind Chang liver cells nonspecifically (data not shown).

We next sought to examine whether G10 would prevent the binding of HBV virions to cells. Culture supernatant containing HBV virions (10^4 copies) secreted from HepG2 2.2.15 was preincubated with serially diluted anti-pre-S1 scFv from G10 or 1E4, and then reacted with Chang liver cells. Binding of virions to the cells was inhibited by G10 and 1E4 in a dose-dependent manner (Fig. 3D). G10 had greater inhibitory activity than 1E4. Although anti-pre-S1 G10 completely neutralized HBV binding to Chang liver cells at 100 μg/ml, G10 could block HBV binding by 90% at 10 μg/ml and by 50% even at 1 μg/ml.

4. Discussion

In this study, we described the selection of functional scFvs being able to neutralize the binding of HBV pre-S1 protein and HBV virions to Chang liver cells using a large naive human scFv library containing 1.1×10^{10} clones. Many reports indicated that naïve libraries with more than 10^{10} clones may have antibodies with moderate to high affinities against a wide variety of antigens (Loset et al., 2005; Sblattero and Bradbury, 2000; Sheets et al., 1998). For construction of naïve antibody library, we used total RNAs from PBMCs of multiple donors to avoid the bias in the diversity introduced by the donor's recent immune history and major differences in mRNA contents (Vaughan et al., 1996; de Haard et al., 1999). We employed the scFv format because the expression level of scFv in *E. coli* is typically higher and the construction of library is simpler than that of Fab. However, it has been known that scFvs have the propensity to form oligomeric structures such as dimer or trimer (Power et al., 2003), which may lead to multivalent interactions with target molecules, causing unexpected high avidity. To circumvent this problem related with oligomeric configuration of scFv to endow them with stability and effector functions including complement-mediated lysis, scFv can be converted into complete antibody by linking scFv to human Fc region (Boel et al., 2000; Kausmally et al., 2004).

To obtain and characterize phage antibodies with desirable affinities and specificities, we utilized a BIAcore for selecting and screening the library and a flow cytometry for analysis of inhibition of binding (IOB) assay. The advantages of BIAcore in panning of antibody library are that it can analyze and select peptides and proteins on the basis of binding kinetics in real time (Malmborg et al., 1996), and the panning process using BIAcore is much faster than conventional biopanning methods: four rounds of biopanning typically take at least 2 weeks to perform, whereas flow-based selection can be performed in a single day. As for the flow cytometry assay, although it is not efficient at detecting an internalized protein or virus, it appears to be very effective means to quantify protein binding to the cell surface. In addition, the inhibitory activity of each antibody fragment could be compared directly.

Following selection and screening of the naive library, we obtained two phage antibodies (Table 1) of which affinities were 1.69×10^{-7} and 1.12×10^{-6} M and the off-rates were 2.05 and 5.56×10^{-3} s^{-1}. Previously others and we reported the construction of human Fab or scFv library derived from immune donors and demonstrated that the affinities of anti-pre-S1 Fab or scFv antibodies ranged from 10^{-6} to 10^{-8} M (Choi et al., 1998; Zhang et al., 2004). The comparison of G10 with those antibodies indicates that the naïve human antibody library we have developed has as sufficient specificities and affinities as immune libraries. The antibody dissociation rate depends on several different factors including flow rate, buffer composition, and antigen concentration on the surface. Therefore, altering stringency by changing the conditions that affect the dissociation kinetics of the flow-based system can make it possible to select scFvs that have greater affinities and desirable binding kinetics using BIAcore.

Data from previous studies suggested that pre-S1–hepatocyte interaction is essential for HBV attachment and infection (De Meyer et al., 1997; Le Seyec et al., 1999) and that pre-S2 contributes to cell attachment (Gerlich et al., 1993). In this study, we used pre-S1 and pre-S2 produced from *E. coli*, which were not glycosylated. The finding that pre-S1, not pre-S2, bound to Chang liver cells suggests that glycosylation is essential for pre-S2, not pre-S1, to bind to liver cells.

The observation that anti-pre-S1 G10 could neutralize HBV virion binding to Chang liver cells suggests that the this antibody may be a candidate for passive immunization in conjunction with hepatitis B immunoglobulin (HBIG) that has been used to prevent acute HBV infection and recurrent infection after liver transplantation. At this time, it would be very prudent that we should consider many technical and practical parameters to develop this antibody as an effective immunotherapeutics; such as affinity improvement which can be facilitated by employing error-prone PCR, chain shuffling or CDR mutagenesis of variable regions of G10 (Schaaper, 1988; Daugherty et al., 2000), animal study using chimpanzees which may strengthen the notion of therapeutic potential of G10, and structural stability which may have the antibody maintained for a desirable period in vivo. In addition, the new phage library we have developed in this study would be a valuable source of antibodies to essentially any viral or cancer cell targets.

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