Abrogation of hepatitis C virus NS3 helicase enzymatic activity by recombinant human antibodies

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The hepatitis C virus (HCV) NS3 protein possesses both protease and helicase activities and is essential for virus replication and maturation. Specific inhibition of NS3 enzymatic activity can be achieved by antibody binding. Transduction of hepatocytes with encoding cDNA leading to intracellular expression of antibody fragments is expected to terminate HCV replication in infected cells. The objective of the present study was the generation of human antibody fragments that neutralize the viral NS3 helicase activity for gene therapeutic applications and drug design. A human immunoglobulin phage-display library cloned from bone marrow aspirate of patients infected with HCV was used for affinity selection against HCV NS3 helicase. Antibody fragments with high affinity to HCV helicase were isolated. To evaluate the inhibitory potential of isolated single-chain antibody fragments, a helicase-mediated, DNA-unwinding enzymatic assay was developed in ELISA format. Recombinant protein comprising the full-length HCV NS3 helicase domain was expressed in the baculovirus expression system. Recombinant antibodies that inhibit the HCV helicase at nanomolar concentrations, with efficacies ranging from 20% to complete abrogation of enzymatic unwinding activity, were identified. These antibody fragments may be useful for novel gene therapeutic strategies that employ intracellular immunization and may provide new insights into the design of small molecule inhibitors of essential HCV proteins.

INTRODUCTION

The hepatitis C virus (HCV) positive-stranded RNA genome encodes a single polyprotein of about 3000 aa. This polyprotein precursor is cleaved co- or posttranslationally by host or viral proteases to yield functional viral proteins (Bartenschlager et al., 1994; Grakoui et al., 1993; Hijikata et al., 1993). The putative structural proteins include the core protein (C) and two envelope proteins (E1 and E2), whereas the nonstructural proteins (NS2, NS3, NS4 and NS5) are believed to be components of a complex responsible for viral RNA replication (Bartenschlager et al., 1994; Steinkuhler et al., 1996; Suzich et al.; 1993; Tomei et al., 1993).

HCV NS3 is a multifunctional enzyme with three known catalytic activities segregated into two distinct domains. The serine protease activity resides in the N-terminal one-third of the protein. The nucleoside triphosphatase and helicase activities are localized in the remaining C-terminal domain (Gwack et al., 1997; Kim et al., 1997; Kolokhlov et al., 1994; Preugschat et al., 1996). HCV NS3 has been reported to be processed internally (Shoji et al., 1999; Yang et al., 2000). The crystal structure of NS3 helicase has been resolved (Cho et al., 1998; Kim et al., 1998; Yao et al., 1997). Helicase activity is reported to be necessary for RNA unwinding during the replication of the viral genome (Gwack et al., 1997; Wardell et al., 1999). Therefore, the HCV NS3 helicase is a promising target for HCV-specific drug therapy. Recently, a dimer model for NS3 helicase was proposed based on crystal structure and mutational analysis of the NS3 helicase (Cho et al., 1998; Khu et al., 2001). This provides new opportunities for designing compounds that inhibit dimerization specifically. Such inhibitors will be specific for the enzyme, as the dimer structure of the HCV helicase is unique (Khu et al., 2001).

Intracellular antibodies (intrabodies) with inhibitory properties represent a new class of neutralizing molecules with great potential for gene therapy. Single-chain antibody fragments (sFvs) have been shown to possess favourable properties as therapeutic agents. These recombinant antibodies consist of the variable domains of heavy (VH) and light (VL) chain connected by a polypeptide linker, generating a fully functional antigen-specific binding unit (Chen et al., 1994a; Marasco, 1997). The variable domains are expressed as a single polypeptide chain, increasing the

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stability of sFv fragments in comparison to complete immunoglobulins, e.g. in the reducing environment of the cytosol (Schouten et al., 2002). Recombinant antibody technology (phage display) permits the generation of high-affinity antibodies that are highly specific for the target protein (Gram et al., 1992; Hoogenboom & Winter, 1992; Rader & Barbas, 1997; Winter et al., 1994). Combinatorial libraries of immunoglobulin genes can be expressed on the surface of filamentous bacteriophages, thus colocalizing the antibody protein with its encoding DNA sequence within the phage (Marks et al., 1991; McCafferty et al., 1990; Pope et al., 1996). Phages displaying antibodies with high affinity against the target molecule are highly enriched during several rounds of affinity selection and reamplification (panning). SFvs with inhibitory properties against the target protein can be expressed directly inside cells, creating an active molecule for protein-based gene therapy. The employment of human SFvs should minimize the immune response against the foreign protein (Chen et al., 1994a; Marasco, 1997; Rader & Barbas, 1997). The clinical evaluation of two human monoclonal antibodies (mAbs) binding to hepatitis B virus demonstrated that intravenous administration of these recombinant antibodies was well tolerated (Galun et al., 2002).

Recently, the isolation and characterization of a murine mAb that inhibits HCV RNA-dependent RNA polymerase was described (Moradpour et al., 2002). In the present study, we report the generation of human sFv antibody fragments that bind to the HCV NS3 helicase, another enzyme essential for the HCV replication cycle. We show efficient neutralization of the helicase unwinding activity upon binding of high-affinity sFv antibodies.

METHODS

Recombinant proteins. Complete recombinant HCV NS3 protein, expressed in *Escherichia coli* and purified under denaturing conditions, was obtained from Mikrogen. According to the manufacturer, the NS3 protein is 527 aa in length (70 kDa) and contains aa 1007–1534 of the coding region. The recombinant His₆-tagged NS3 helicase domain was produced in insect cells using the Bac-to-Bac Baculovirus Expression system (Gibco-BRL). The helicase-encoding sequence was amplified using the following primers: 5’-TAGGGATCCGAGATCCCCGGTGTTCACG-3’ and 5’-CGGGCGGCCGGC-GAATTATTACGTCGACCTCCAGTCCAGGAGGC-3’, containing BamHI and NotI restriction sites, respectively. Plasmid pBRTM-HCV was used as a template (Grakoui et al., 1993). The amplified fragment was digested with BamHI and NotI, cloned into pFastBacHTa and transformed into MAX Efficiency DH10Bac *E. coli* cells. Recombinant Bacmid was isolated, treated with CellFECTIN (Gibco-BRL), according to manufacturer’s instructions, and transfected into SF21 insect cells. SF21 cells were grown at 27 °C in supplemented Grace’s Insect medium (Invitrogen) containing 10% heat-inactivated FCS (Biochrom) and 10 μg gentamicin ml⁻¹. Recombinant baculovirus was collected 96 h after transfection, resuspended in SF21 cells and used to infect High Five insect cells (Invitrogen) for recombinant protein production. High Five insect cells were propagated in Ultimate Insect Serum-Free medium (Invitrogen) as an adherent cell culture and were harvested 4 days after infection. Approximately 10⁹ cells were resuspended in 10 ml lysis buffer (50 mM NaPO₄, 0·3 M NaCl, 10 mM β-mercaptoethanol, 20% glycerol, 2% TritonX-100, 0·5% Nonidet P-40, 1 mM PMSF, 4 µg leupeptin ml⁻¹, pH 8) and disrupted by sonication (250 W, 40 kHz, four strokes of 30 s with intervals of 30 s on ice). The supernatant obtained by centrifugation at 12 000 g for 30 min was mixed with 0·5 ml Ni-NTA agarose (Qiagen) and incubated shaking for 1 h at 4 °C. The lysate/Ni-NTA mixture was loaded onto a column and washed with 20 ml lysis buffer containing 20 mM imidazole. The helicase–His₆-tagged fusion protein was eluted with 200 mM imidazole in lysis buffer without protease inhibitors and β-mercaptoethanol, and dialysed overnight against 50 mM NaPO₄, 0·3 M NaCl, 20% glycerol and 0·5% TritonX-100, pH 8.

Human sFv library and affinity selection. The cloning of a combinatorial sFv library from patients chronically infected with HCV has been described previously (Tesmann et al., 2002). Briefly, human V₃ and V₄ fragments derived from bone marrow aspirate were cloned into the phagemid vector pAK100 (Krebber et al., 1997). This phage-display vector allows the expression of an sFv as a gene III fusion protein at the surface of filamentous phages. N-terminal FLAG and C-terminal c-Myc tag sequences were used for the immunodetection of the sFv antibody fragments.

Library growth, phage preparation and soluble antibody expression were carried out as described (Harrison et al., 1996). Phages were prepared by infecting the library stock with VCS M13 helper phage (Stratagene). A phage solution of 10¹⁵ to 10¹⁶ transducing units diluted in PBS containing 2% BSA was incubated in microtitre plates coated with approximately 200 ng recombinant NS3 helicase for 2 h at room temperature. The microtitre plate was washed extensively (20 times) with PBS containing 0·1% Tween-20 to remove nonspecifically bound phages. Subsequently, phages bound to NS3 helicase were eluted with 100 mM triethylamine and neutralized immediately with equal amounts of 1 M Tris/HCl, pH 7·4. Eluted phages were used for refection of the suppressor *E. coli* strain XL-1 Blue for phage propagation and for subsequent rounds of selection. The panning procedure was repeated three times. Soluble expression and screening were carried out using the *E. coli* nonsuppressor strain HB2151. Antibody purification and ELISA were described previously (Tesmann et al., 2002).

Sequencing and V gene alignment. Custom sequencing was carried out by MWG Biotech. The alignment of the nucleotide sequences of rearranged V genes to their closest germline V, D and J segments was performed according to Kabat et al. (1991) and Martin (1996). Multiple sequence alignment was performed usingCLUSTALX (Higgins et al., 1996).

Determination of equilibrium dissociation constants. Biomolecular interactions were measured by surface plasmon resonance (SPR) using BIACORE 2000 (BioSensor) in HBS-EP buffer [150 mM NaCl, 3·4 mM EDTA, 0·05% (v/v) surfactant P20, 10 mM HEPES, pH 7·4]. The purified NS3 helicase protein was immobilized on a CM5-r sensor chip using the Amine Coupling kit (BioSensor). Sample protein (sFv antibodies) was diluted in Hanks’ balanced salt buffer and injected at different concentrations at a flow-rate of 30 μl min⁻¹. The surface was regenerated with 10 μl 30 mM HCl between sample injections. Off- and on-rates were determined using the BIAC evaluation 3.0 software (BioSensor), assuming a single-step monovalent interaction.

NS3 helicase-mediated DNA-unwinding assay. The helicase activity assay was developed in ELISA format. The substrate was composed of two annealed complementary DNA strands: the 5'–biotin-labelled template strand (5’–GGTTTTAAATAGGGAGGA-CACCGTCTGACTGGAAAACTCCCCGGGTACCGAGTCG-3’)
and the 5′-digoxigenin-labelled release strand (5′-GGTTTCCCCAGTGAGCAGGTTGTC-3′) (MWG Biotech). The annealing of two oligonucleotides at a molar ratio of 1:3 (template:release) was carried out in 10 mM Tris/HCl, 25 mM NaCl and 1 mM EDTA, pH 7.6, by heating to 100 °C for 10 min followed by cooling slowly to room temperature. High-binding, polystyrene half-area microtitre plates (Costar) were coated with 10 μg streptavidin ml⁻¹ (Fluka) and blocked with 2% BSA in PBS. The helicase substrate (concentration of the template strand, 0-4 pmol per well, 100 μl) was transferred to the preblocked plate and incubated for 1 h at room temperature. After extensive washing with buffer M (25 mM MOPS, 2 mM DTT, 3 mM MgCl₂), HCV helicase was added in buffer M supplemented with 2.5 mM ATP and 20 pmol per well of the capture oligonucleotide 5′-ACACGTCGTAACAGGAC-3′, which is complementary to the release strand (helicase reaction buffer). After incubation at 37 °C for 1 h, the plate was washed six times with PBS. Release strand not unwound by helicase was detected with alkaline phosphatase conjugated to an anti-digoxigenin Fab-fragment from sheep (Roche), followed by p-nitrophenylphosphate substrate. Absorbance was measured at 405 nm after 30 min of incubation at room temperature.

Calculation of the fraction of bound helicase. The fraction of complexed helicase was calculated assuming a one-step monovalent interaction that is described by the following law of mass action:

\[
K_D = \frac{[\text{helicase} \cdot \text{sFv}]}{[\text{helicase} \cdot \text{sFv}]}\]

where [helicase] is the equilibrium concentration of free helicase, [sFv] is the equilibrium concentration of free sFv, [helicase::sFv] is the equilibrium concentration of helicase-sFv complex and \(K_D\) is the equilibrium dissociation constant.

Where \(K_D\) was derived from the rate constants determined by SPr:

\[
K_D = \frac{k_{off}}{k_{on}}
\]

The fraction of helicase that is complexed by sFv:

\[
I = \frac{[\text{helicase} \cdot \text{sFv}]}{[\text{helicase}] \cdot [\text{sFv}]} = \frac{[\text{helicase} \cdot \text{sFv}]}{[\text{helicase}] \cdot [\text{sFv}] K_D}
\]

Where the unknown equilibrium concentrations are replaced by the known total concentrations (\([\text{helicase}]\) of sFv and helicase:

\[
[\text{helicase}] = c_0(\text{helicase}) - [\text{helicase} \cdot \text{sFv}]
\]

\[
[sFv] = c_0(\text{sFv}) - [\text{helicase} \cdot \text{sFv}]
\]

RESULTS

Expression of recombinant NS3 helicase and enzymatic activity assay

The HCV NS3 helicase domain was cloned into a baculovirus expression vector and expressed as an N-terminal His₆-tagged fusion protein in insect cells. The infection of High Five insect cells with the recombinant baculovirus resulted in the synthesis of a polypeptide with the expected molecular mass of 51 kDa, which could be detected in lysed cells by Tetra-His antibodies (Fig. 1A). Baculovirus-expressed helicase was purified on Ni-NTA agarose in a native form (Fig. 1B).

To determine the enzymatic activity of recombinant helicase, an activity assay in ELISA format was developed (Fig. 2A), based on the principle described by Hsu et al. (1998) with the following modifications. A partly double-stranded oligodeoxyxynucleotide was used as substrate because NS3 helicase was shown to be effective in unwinding both DNA and RNA duplexes (Levin & Patel, 2002; Pang et al., 2002). The substrate was composed of two annealed, partly complementary oligonucleotides, a 5′-biotin-labelled template strand and a 5′-digoxigenin-labelled release strand, and captured onto streptavidin-coated microtitre plates. The helicase reaction was performed in the presence of an excess of capture oligonucleotide complementary to the release strand. This prevented the reannealing of the unwound duplex and improved the efficiency of the assay. Release strand that was not unwound by helicase was detected by anti-digoxigenin antibodies and a subsequent colorimetric reaction.

The recombinant baculovirus-expressed helicase was investigated for its ability to unwind a DNA duplex in the established assay. The percentage of substrate unwinding was determined using standards of the release strand annealed at varying concentrations to the template. The results show that the amount of unwound DNA increases with increasing helicase concentrations in a dose-dependent manner and a saturation corresponding to 100 % unwinding is achieved at approximately 1 μg enzyme ml⁻¹ (Fig. 2B).

Generation of human antibodies by phage display and affinity measurements

The human sFv library, derived from bone marrow aspirate of chronically infected patients and cloned into a phage-display vector (Tessmann et al., 2002), was used for affinity selection. Previously, we described isolation of high-affinity sFv antibodies binding to denatured NS3 helicase.
In the present study, we carried out affinity selection (panning) against an enzymatically active NS3 helicase domain expressed in insect cells. The rationale behind this strategy was that the use of a biologically functional enzyme as a template for panning would allow us to select antibodies against conformational epitopes not represented in the denatured protein. After three rounds of affinity selection, a number of clones which bound strongly...

**Fig. 2.** HCV helicase unwinding activity assay. (A) Schematic representation of a helicase ELISA. Partly complementary, duplex substrate composed of a biotinylated template and a digoxigenin-labelled release strand is immobilized onto streptavidin-coated microtitre plates. The unwinding reaction is carried out by the HCV NS3 recombinant helicase. Unwound release strand is captured by a complementary unlabelled capture oligonucleotide to prevent reannealing of the substrate and is then washed out. The remaining duplex is detected by anti-digoxigenin antibodies conjugated to alkaline phosphatase. (B) Effect of enzyme concentration on substrate unwinding. Helicase reactions were carried out with varying amounts of the recombinant baculovirus-expressed NS3 helicase protein under standard conditions, as described in Methods. Data are expressed as the means of triplicates ± SEM.

(Tessmann et al., 2002).
to the target protein was isolated. Some clones were shown to possess identical DNA sequences, thus encoding the same antibody fragment. Distinct clones, designated sFvs 6, 20, 41, 44, 42, 52 and 59, were chosen for further characterization. The subgrouping of the variable domains and identification of the closest germline V, D and J segment counterparts (Table 1) was performed by alignment using the V Base database (Cambridge, UK) and immunoglobulin germline V genes (NCBI). The deduced protein sequences of the selected antibodies are shown in Fig. 3. An unusual and interesting feature of antibody fragment sFv 6 has been identified. The CDR-H1 and CDR-H3 regions of this clone each contain a single cysteine. Cysteines are not found in the germline genes of the VH exons and only paired cysteines are found in the exons for D2 elements. The closest D segment identified for sFv6 is D3-22/D3-10, in which the cysteines are missing. Thus, both cysteines must have been acquired during affinity maturation. Interestingly, the two cysteines are in close vicinity, allowing the formation of a disulfide bond connecting CDR-H1 and CDR-H3. Alternatively, free cysteines may be a requirement for binding of sFv 6 to helicase.

Affinities of the sFvs were determined by SPR. The HCV helicase was coupled covalently to a layer of carboxymethylated dextran covering the gold surface of a sensor chip. Association kinetics were obtained by monitoring the real-time change of the SPR signal upon injection of the sFv antibody. The dissociation of the bound sFv can be followed when running buffer (HBS-EP) passes along the surface. An example of a sensorgram generated with the sFv antibody. The dissociation rate constants were determined by fitting the sensorgrams to mathematical descriptions of one-step monovalent binding models (BIAevaluation software, BioSensor). The equilibrium dissociation constant is represented by the ratio of dissociation rate to association rate (Table 2).

sFvs 6, 20, 41, 44, 42 and 59 showed high affinities to NS3 helicase, with dissociation equilibrium constants between $10^{-8}$ and $10^{-9}$ M. sFv 52, although producing a strong positive signal in ELISA, did not bind to helicase protein immobilized covalently onto the sensor chip. Therefore, the helicase epitope recognized by sFv 52 seems to be either inaccessible or destroyed (e.g. through coupling). Moreover, when sFv 52 was bound to an Ni-NTA sensor chip via its His$_6$-tag, injection of an untagged helicase protein led to elution of the antibody from the Ni-NTA surface. Thus, binding of sFv 52 to helicase is incompatible with binding of its His$_6$-tag to the Ni-NTA surface, indicating steric hindrance or conformational changes caused by antigen binding (data not shown).

### Inhibition of NS3 helicase by recombinant antibodies

To assess the inhibitory potential of selected sFvs by DNA-unwinding assay, we used both antibody and helicase proteins purified by Ni-NTA (Fig. 1B). The sFv antibodies were preincubated with the enzyme in reaction buffer, the helicase reaction was performed and the fraction of unwound substrate was determined. During initial screening, we identified seven recombinant antibodies that inhibited helicase-mediated DNA unwinding at nanomolar concentrations, with efficacies ranging from 20% to complete abrogation of helicase activity (Fig. 5). sFv 1-2, selected against denatured NS3 protein, was able to neutralize the helicase reaction, but all other inhibitory antibodies were obtained from selection against enzymatically active helicase (sFvs 59, 42, 6, 20, 44 and 41).
a similar heavy chain), which could be tested only at lower concentrations (up to 30 nM) because of poor expression in *E. coli*, still showed inhibitory effects.

**DISCUSSION**

Antibody fragments expressed intracellularly represent a new class of therapeutic molecules for gene therapy. They can be designed to bind and inactivate target molecules, such as viral enzymes inside infected cells, thereby interfering with virus replication. Even fully reshaped and humanized mAbs of rodent origin may result eventually in an anti-immunoglobulin response in at least a proportion of patients (Routledge et al., 1993). To minimize immunogenicity, antibodies of human origin should be used for therapy. Therefore, phage display was chosen to isolate human mAb fragments against the HCV helicase, an enzyme that is essential for virus replication. Affinity selection from the cloned immune library against the enzyme yielded an antibody subpopulation of high-affinity binders, representing distinct subfamilies of antibody germline genes. In addition to the germline gene diversity observed, unusual codons were found in the sequences encoding the hypervariable loops of the antibody heavy chains. These loops are likely to be a consequence of a somatic mutation process during affinity maturation.

**Fig. 3.** Deduced amino acid sequences of human sFv antibodies selected against enzymatically active HCV NS3 helicase protein.
The antibody–antigen interaction studied by SPR and evaluated by fitting the results to mathematical models revealed different kinetics parameters of the selected clones, with association rate constants between $8.7 \times 10^4$ and $8.4 \times 10^5$ M$^{-1}$ s$^{-1}$ and dissociation rate constants between $7.1 \times 10^{-4}$ and $1.6 \times 10^{-3}$ s$^{-1}$, resulting in apparent equilibrium constants ranging from $2.5 \times 10^{-9}$ to $4.4 \times 10^{-8}$. In some cases, we observed deviations from the simple monovalent one-step binding model, which can be caused by the presence of dimeric or multimeric structures. Moreover, it has been observed that only a small fraction of about 15% of the immobilized helicase was recognized by different antibodies. This might be an indication that immobilization of the helicase led to partial, and for sFv 52, to complete, inactivation or masking of a binding epitope.

It has been reported that NS3 helicase may exist as a homodimer in vivo (Khu et al., 2001). Because of the coupling procedure used, the immobilization of the enzyme is undirected and any of the exposed lysine residues can be modified. This may affect the capability of dimerization. Alternatively, immobilization may arrest helicase conformationally and might affect binding of antibodies that require helicase to undergo a conformational change either before or upon binding. Taken together, kinetics analysis indicates differences in antibody–antigen interaction modes among the cloned sFvs and future studies will aim to resolve the underlying mechanisms of binding and inhibition of helicase activity. The affinity data obtained by SPR measurements are in good agreement with the individual inhibition rates of the sFvs, which were determined with the established enzymatic activity assay. This assay allows the reproducible quantification of helicase unwinding activity and provides a rapid screening method for inhibitory compounds. We report efficient inhibition of HCV HS3 helicase in vitro at nanomolar concentrations of the sFvs. To date, no intracellular system for monitoring the helicase activity of HCV in vivo has been described. An option for the investigation of the inhibitory properties of

Table 2. Kinetic rate constants of the sFv antibodies determined by SPR

<table>
<thead>
<tr>
<th>sFv clone</th>
<th>Association rate constant ($k_a$, M$^{-1}$ s$^{-1}$)</th>
<th>Dissociation rate constant ($k_d$, s$^{-1}$)</th>
<th>Dissociation equilibrium constant ($K_D$, M)</th>
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<td>42</td>
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<td>$3.1 \times 10^{-4}$</td>
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<td>41</td>
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<td>$2.1 \times 10^{-3}$</td>
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<tr>
<td>44</td>
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<tr>
<td>59</td>
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antibodies expressed intracellularly could be a new HCV replicon system, which has been developed recently for the assessment of functions of HCV nonstructural proteins (Lohmann et al., 1999). A similar HCV replicon was constructed by Pflugheber et al. (2002) to examine the role of the HCV NS5 protein in disrupting a host antiviral pathway. Intracellular immunization with inhibitory antibodies in a cell line harbouring a HCV replicon may be a next step for the target evaluation. This could be used to assess the efficiency of inhibition of virus replication via intracellular inactivation of one of the viral enzymes, e.g. the helicase, and may add to our knowledge of designing improved HCV inhibitory drugs.

Inhibitory effects of sFv fragments used as intrabodies have been shown with various intracellular target proteins. Various intrabodies were shown to be effective against human immunodeficiency virus replication and infection (Marasco et al., 1999; Mhashilkar et al.; 1999; Poznansky et al., 1998; Kitamura et al., 1999; BouHamdan et al., 1999; Goncalves et al., 2002). Other intrabodies were used successfully against various oncoproteins (Jean et al., 2000; Deshane et al., 1995; Chen et al., 2002).

To achieve antiviral effects against HCV, the cDNA encoding inhibitory antibodies have to be transduced into hepatocytes (reviewed by Wu et al., 2002). Successful transduction has been demonstrated with, for example, retrovirus and lentivirus vectors (BouHamdan et al., 1999; Chen et al., 1994b; Marasco et al., 1999; Richardson et al., 1998). To date the first clinical studies involving patients with ovarian cancer are ongoing using adenovirus vectors to express anti-erbB-2 intrabodies (Alvarez et al., 2000; Deshane et al., 1995). Using the appropriate gene transfer vectors, intracellular immunization with intrabodies could be a promising approach for treatment of chronic HCV infection.

Once sFv fragments have been isolated, the cloning and expression of complete human IgG, IgA or IgM antibodies is possible (Boel et al., 2000). Production of recombinant antibodies in a heterologous system, such as transgenic yeasts or plants, is simple and cheap in comparison to the isolation of antibodies from the sera of HCV-infected patients. Moreover, there is no risk of infection with HCV or other human pathogens with the use of recombinant high-affinity antibodies for diagnostic and therapeutic purposes.

Further experiments will examine the molecular mechanism of the antibody-mediated enzyme inhibition. This information may provide a structural basis for the molecular mimicry of antibody recognition loops and may aid in the rational design of small molecule inhibitors of essential viral proteins.

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**Fig. 6.** Inhibition of HCV helicase enzymatic activity. (A) Dose-response curves with increasing concentrations of sFvs are shown. Data are expressed as the means of two independent experiments, each in duplicate, where the SEM values were within 10%. ◆ sFv 59; ■ sFv 6; △ sFv 20; × sFv 52; ⋄ sFv 42. (B) Predicted fractions of complexed helicase based on antibody affinities.
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immunodeficiency virus replication and growth advantage of CD4+ T cells from HIV-infected individuals that express intracellular antibodies against HIV-1 gp120 or Tat. *Hum Gene Ther* 9, 487–496.


