An Antibody Fragment from a Phage Display Library Competes for Ligand Binding to the Low Density Lipoprotein Receptor Family and Inhibits Rhinovirus Infection*

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Recently antibodies with a wide range of binding specificities have been isolated from large repertoires of antibody fragments displayed on filamentous phage, including those that are difficult to raise by immunization. We have used this approach to isolate an antibody fragment against chicken very low density lipoprotein (VLDL) receptor. It binds to the receptor with good affinity ($K_{d} = 2 \times 10^{8} \text{m}^{-1}$) as measured by plasmon surface resonance, and competes for binding of natural ligands (vitellogenin, VLDL, and receptor-associated protein). The antibody also binds to other members of the low density lipoprotein (LDL) receptor family including rat LDL receptor and human and rat low density lipoprotein receptor-related protein (LRP/2MR), and it competes for binding of receptor-associated protein to LRP/2MR. Moreover, the antibody fragment inhibits infection of human fibroblasts deficient in LDL-R but expressing LRP/2MR by human rhinovirus. Binding of the antibody is abolished upon reduction of the antibody thus recognizes the ligand binding site(s) of several members of the LDL receptor family, in contrast to antibodies produced by hybridoma technology.

The low density lipoprotein (LDL) receptor of mammals is the prototype of a family of related proteins. Members of the LDL receptor family have several structural modules in common; (i) “binding repeats,” complement-type domains consisting of 4–9 residues displaying a triple disulfide bond-stabilized negatively charged surface (head-to-tail combinations of these repeats are believed to specify ligand interaction); (ii) epidermal growth factor precursor-type repeats, also containing six cysteines each; (iii) modules of 50 residues with a consensus tetrapeptide, Tyr-Trp-Thr-Asp (YWTD); and (iv), in the cytoplasmic region, signals for receptor internalization via coated pits, containing the consensus tetrapeptide Asn-Pro-Xaa-Tyr (NPXY).

The LDL receptor family includes at least 4 proteins; the LDL receptor (LDL-R), the low density lipoprotein receptor related protein (also termed LRP/2MR), gp330 (also termed megalin), and the very low density lipoprotein (VLDL) receptor. The LDL receptor (LDL-R) has a cluster of 7 binding repeats and binds to apolipoprotein B (apoB) and apolipoprotein E (apoE) (1, 2). LRP/2MR is a giant receptor (4525 amino acids) containing 4 clusters of 2 to 11 binding repeats and has many ligands including apoE (3), apoM-proteinase complexes (4, 5) among others (4–12), and a 39-kDa intracellular protein (receptor-associated protein or RAP). RAP binds to LRP/2MR with high affinity, co-purifies with LRP/2M from liver and placenta (13, 14), and competes for binding with all known LRP/2MR ligands (4, 7–9, 15, 16). Whereas the majority of the ligands bound by LRP/2MR fail to be recognized by LDL-R, human rhinoviruses (HRVs) of the minor receptor group type attach to either of these proteins (8). Recently, it was shown that RAP also binds to LDL-R but with much lower affinity than to LRP/2MR (17). Gp330 (megalin) is a membrane glycoprotein (the Heymann nephritis antigen in rats) (18), is closely related to LRP/2MR in structure (19), and binds to many of the same ligands (except for apoM-proteinase complexes) (20). The VLDL receptor (VLDL-R) is characterized by a cluster of 8 binding repeats, and binds VLDL and other apoE containing lipoproteins (21).

The LDL receptor family is also present in birds; for example, in the laying hen the chicken LDL-R (22) and an LRP/2MR (23) are expressed in somatic cells, and an LRP/2MR-like protein (380 kDa) and a receptor (OVR) for very low density lipoprotein, vitellogenin (VTG) (24), and apoE-containing lipoproteins (25) are expressed in oocytes. The binding specificity and sequence of OVR indicate that it is the chicken homologue of VLDL-R (26).

Although polypeptides of the LDL receptor family are highly related, and most of the known receptors bind to apoE, it has proved difficult to map the ligand binding sites with respect to...
different ligands. The sites are thought to comprise the cysteine-rich binding repeats, and to involve carboxylate residues on the receptor and lysine and arginine residues on the ligand (2); in apoE most of the positive charges are clustered to one side of the protein (27). Although LDL-R binds to few ligands, LRP/α-MR (3–12), gp330 (20), and OVR bind to a wide spectrum of ligands with high affinity (24, 25, 28). In LRP/α-MR it appears that the different ligands bind to different clusters of binding repeats (29, 30), but OVR and LDL-R contain only one cluster of binding repeats, and more subtle differences must therefore dictate their ligand binding properties.

Mapping of the ligand binding sites has been hampered by the difficulty of raising blocking antibodies by immunization. Recently the display of repertoires of antibody fragments on the surface of filamentous bacteriophage, and the selection of antigen-binding phage (31), has provided a means of making antibodies without immunization (32). Antibody repertoires can be derived from the rearranged V-genes of populations of lymphocytes (32, 33) or from V-gene segments rearranged in vitro (34–36). Antibodies with many different specificities have been isolated from the same repertoire, including some directed against self-antigens (33) and highly conserved proteins (36). Although the binding affinities of the antibody fragments were often moderate, it has been possible to obtain antibodies with good binding affinities (K_{aff} = 10^{-10} M^{-1}) from very large repertoires (34). Here we used a large phage antibody repertoire to isolate antibody fragments against OVR.

LDL-R and LRP/α-MR both serve as receptors for one group (minor group) of HRVs (8), the main causative agents of the common cold. Due to the large number of different serotypes, vaccination is not possible; therefore, other means of preventing or curing the common cold are being thought of, including inhibition of virus-specific enzymes (37), or blockage of the viral receptors (38, 39). In this article we show that viral infection can be blocked with the single chain antibody fragment described.

MATERIALS AND METHODS

Animals and Diets—White Leghorn laying hens were purchased from Heinld (Vienna) and maintained as described (40). Roosters (20–30-week-old) were treated with 17-ethynylestradiol dissolved in propylene glycol, by injecting 10 mg/kg body weight into the breast muscle. After 72 h, blood was collected from the jugular vein and mixed with the following additives giving the indicated final concentrations (10 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 5 mM leupeptin, 0.1 mM aprotinin).

Preparation of Oocyte Membranes and OVR—Logenic follicles (4–6 mm diameter) excised from mature laying hens according to Ref. 10. Oocyte membranes were prepared from previtellogenic membranes (Hybonder-C, Amersham) in 20 mM Tris-HCl, 0.15 M glycin buffer, pH 8.4, for 90 min at 200 mA. After transfer, proteins were visualized by staining the membrane with Ponceau S (2 gliter in 100% (v/v) trichloroacetic acid) and rinsing with water. Nitrocellulose membranes used for Western blot analysis were blocked with 5% non-fat dry milk in PBS containing 0.1% Tween 20. Bound rabbit antibodies were detected with horseradish peroxidase-conjugated protein A (Amersham) and enhanced chemiluminescence system (ECL, Amersham, Arlington Heights, IL). The antibody fragment was detected using anti-myc antibody 9E10 and anti-mouse IgG conjugated with alkaline phosphatase (Promega) (34). Binding specificity was tested by competition of soluble V-genes from the various incubations IgG fractions prepared by protein-A affinity chromatography were used. Antiserum against human LRP/α-MR was kindly provided by Dr. J. Gliemann of the University of Aarhus, Denmark.

Electrophoresis and Transfer to Nitrocellulose—SDS-PAGE under nonreducing conditions was performed according to Laemmli (46) on 4.5–12% gradient slab gels at 180 V for 60 min using the mini-gels (Bio-Rad). Molecular sizes of proteins were estimated with Bio-Rad markers (6–200 kDa). Proteins were electrophoretically transferred to nitrocellulose membranes (Hybond-C, Amersham) in 20 mM Tris-HCl, 0.15 M glycin buffer, pH 8.4, for 90 min at 200 mA. After transfer, proteins were visualized by staining the membrane with Ponceau S (2 gliter in 100% (v/v) trichloroacetic acid) and rinsing with water. Nitrocellulose membranes used for Western blot analysis were blocked with 5% non-fat dry milk in PBS containing 0.1% Tween 20. Bound rabbit antibodies were detected with horseradish peroxidase-conjugated pro-tein A (Amersham) and enhanced chemiluminescence system (ECL, Amersham, Arlington Heights, IL). The antibody fragment was detected using anti-myc antibody 9E10 (Stratagene) and rabbit anti-mouse IgG conjugated with horseradish peroxidase, and the membranes were exposed on Reflecter™ film (DuPont) for the indicated times.

Selection of Antibigen Binding Phage by Library Panning—A phage antibody repertoire was prepared as described.2 Briefly repertoires of human heavy and light chain V-genes were amplified from human lymphocytes from tonsils, and then assembled to encode single chain Fv fragments as described in Ref. 32. The assembled V-genes were cloned into the ampicillin-resistant phagemid pCANTAB6 (Cambridge Antibody Technology, Cambridge, UK) to append a COOH-terminal hexa-histidine (His) tag to allow purification of the fragments (47) and a myc-tag (48) to facilitate detection of the fragments. A large repertoire of 1.5 × 10^{12} different phage clones was thereby prepared. In the first round of selection 10^{11} phage were panned using immunobeads (Maxisorb, Nunc) coated with 10 μg purified OVR in 50 mM NaHCO_3, pH 8.6, overnight at 4°C (33). Four additional rounds were carried out in immunobeads coated with 5 μg/ml antigen. Affinity enrichment was carried out as described (34).

Screening and Sequencing of Clones—Phage were isolated from single ampicillin-resistant colonies of infected (suppressor) Escherichia coli TG-1 using helper phage VCS-M13 (Stratagene), and the phage used to infect the (non-suppressor) E. coli HB2151. Single ampicillin-resistant colonies were used to inoculate 200 μl of culture broth in microtiter plates, and the expression of soluble scFv fragments induced by addition of 1 mM isopropl-β-D-thiogalactopyranoside to the cultures (33). Bacteria were pelleted, and the supernatants containing scFv fragments were screened for binding to the antigen by ELISA using the anti-myc-tag antibody 9E10 and anti-mouse IgG conjugated with alkaline phosphatase (Promega) (34). Binding specificity was tested by comparing the signals obtained from plates coated with either 1 μg OVR or 10 μg/ml bovine serum albumin. Soluble fragments giving an ELISA signal at least 7 times higher for OVR than bovine serum albumin were identified, and using specific primers (36) the encoding variable heavy and light chain regions were amplified via polymerase chain reaction from single colonies. The amplified DNA was subjected to cDNA sequencing using standard method using the TAA DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems Inc.) and the PCRHLINK primer (Cambridge Antibody Technology, Cambridge, UK) for the heavy chain region and FQDSEQ1 (34) for the variable light chains. The sequences of the V-genes were compared with a library of V-gene segments using SeqEd (Applied Biosystems Inc.) and MacVector 3.5 (IBI Kodak, New Haven, CT).

Preparation of Monodonal Reagents—For further characterization of the scFv fragments, the infected HB2151 bacteria were grown in 1 liter of 2 × TY medium supplemented with 0.1% glucose and 100 μg/ml ampicillin and were induced overnight with 1 mM isopropyl-β-D-thiogalactopyranoside at 23°C (49). Bacteria were removed using a tangential
PRIMARY SEQUENCE OF scFv7

Heavy Chain

FR1  CDRI  CDR2  CDR3  FR2
QQV5lQEQVQGQWYVRSCGR2ASWQF7  YNGWQWQGQ9SGEM3  LKMK9lQGQWQF7  IYQVTFYTR 5G55 5G55 5G55 5G55 5G55

Light Chain

FR1  CDRI  CDR2  CDR3  FR2  FR3  CDRI  FR4
DIIKQGQSP6LQASISGQITC  RAGElYlBNQMQGQ5G55  5G55 5G55 5G55 5G55 5G55 5G55

FR, framework region; CDR, complementarity-determining region

FIG. 1. Amino acid sequence of scFv7 as deduced from the cDNA sequence. The DNA fragment encoding the heavy and light chain of scFv7 was amplified via polymerase chain reaction from bacterial colonies; DNA obtained was subjected to automatic sequencing. Sequences obtained was compared to published heavy and light chain data. The heavy chain fragment closely resembled DP-7 of the V_{H}1 family, the light chain fragment showed a high degree of similarity to L12a of the V_{L}1 family. Complementarity determining regions are in normal lettering. Amino acid residues different from those present in DP-7 and L12a are indicated in small letters. The peptide (composed of four repeats of GGGS) linking heavy and light chain fragments is not shown.

Isolation of scFvs with Affinity for the OVR from a Phage Library—The repertoire of scFv fragments displayed on a filamentous phage was selected with OVR. The selected phage were grown and subjected to further rounds of selection and growth, in total 5 rounds. After rounds 3 and 5 the soluble scFv fragments from phage-infected colonies were analyzed for binding to OVR by ELISA. Binding was detected for 15 out of 18 clones from round 3. DNA sequencing of the encoded scFv fragments indicated that a single scFv sequence (scFv7) predominated in both rounds 3 and 5 (10 out of 18 clones).

RESULTS

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Specificity of Soluble Antibody Fragment scFv7—We analyzed the specificity of binding of scFv7 to OVR and other members of the LDL-R family by Western blotting. As shown in Fig. 2A (lane 1), scFv7 binds specifically to OVR (95 kDa) in crude oocyte membrane extracts. It also reacts with a species of ~400 kDa present in membrane extracts of chicken liver (lane 2, see arrowhead). As seen by overexposure of lane 1, the same protein was also present in oocyte extract, although in a much lower amount (not shown). This appears to be chicken somatic LRP_{o2}/MR as detected by an anti-peptide antibody directed against the carboxyl terminus (23), and lane 3 shows chicken oocyte LRP as detected by a polyclonal antiserum against OVR and known to cross-react with oocyte LRP (45). This shows that scFv7 binds to chicken somatic LRP, but fail to bind to oocyte LRP on the Western blot. We also analyzed liver membrane extracts from estrogenized rats (41). As shown in lane 5, scFv7 binds rat LRP_{o2}/MR (as detected in lane 7 using an antibody specific for mammalian LRP_{o2}/MR). In contrast, scFv7 hardly recognizes the rat LDL-R (as detected in lane 6 with an antibody specific for LDL-R). However, upon longer exposure, we did detect a faint band in lane 5 (not shown) commigrating with rat LDL-R.

In Fig. 2B, we used detergent extracts from COS-7 cells which had been transiently transfected with a plasmid carry-
ing OVR-cDNA (lane 1) or with a control plasmid (lane 2) (26). On the Western blot, scFv7 detected a strong band in lane 1 that comigrated with an Ooyte membrane extract (lane 3). The weak band seen in the mock-transfected cells (lane 2) and comigrating with the recombinant OVR probably represents the endogenous simian VLDL-R. There is an additional band migrating slightly slower than OVR in lanes 1 and 2. This protein was not further characterized; it might correspond to the simian LDL-R or to another, still unknown member of the LDL receptor family. The higher band (500 kDa) seen in lanes 1 and 2 is most likely simian LRP/α2MR which is abundantly expressed in COS cells (57).

Finally, as shown in Fig. 2C, the antibody strongly discriminates between non-reduced (lane 1) and reduced (lane 2) receptors (OVR migrating at 95 kDa and somatic LRP/α2MR migrating at about 500 kDa, respectively), whereas a control antibody directed against a synthetic peptide derived from the carboxyl terminus of OVR reacted with both forms of OVR equally well, but failed to bind to somatic LRP/α2MR (lanes 3 and 4). Note that OVR migrates with a much higher apparent molecular weight in its reduced form when compared to the migration of its unreduced form (compare lanes 3 and 4).

Kinetic Analyses—ScFvs can form monomers, dimers, and trimers (15%). We obtained IgG with a specific activity of 1.2 × 10^8 cpn/μg on lanes 1 and 2) and nitrocellulose strips were exposed for 16 h. For lanes 3 and 4, a polyclonal antiserum antibody specific for the carboxyl terminus of OVR was used at 10 μg/ml. Bound IgG was detected as described under A. Exposure time was 2 min.

Fig. 2. Western blot of membrane extracts prepared from organs from various species and from cells transfected with OVR-expression plasmid. Electrophoresis was performed under nonreducing conditions on 4.5-12% SDS-polyacrylamide gradient gels. Proteins were electrophoretically transferred to nitrocellulose. The positions of marker proteins with molecular sizes of 116 and 200 kDa and run on the same gels are shown. A: lanes 1 and 3, Triton X-100 extracts of chicken follicle membranes (5 μg of protein/lane); lanes 2 and 4, Triton X-100 extracts of chicken liver membranes (15 μg of protein/lane); lanes 5-7, Triton X-100 extracts of estrogenized rat liver membranes. Nitrocellulose membranes were incubated with scFv7 at 5 μg/ml (lanes 1, 2, and 5), with polyclonal antibody against OVR and oocyte-specific LRP/α2MR at 1 μg/ml (lane 3); with polyclonal antibody against chicken somatic LRP/α2MR at 10 μg/ml (lane 4). Western blotting was carried out using 125I-labeled scFv7 for OVR and a 35S-labeled scFv fragment (lane 6). A sensor chip to which the receptors had been coupled was used immediately after antibody injection. Inhibition of binding of OVR to the sensor chip (1411 RU), and scFv7 injected at four concentrations in the range of 7 × 10^{-8} to 2 × 10^{-6} M. Both monomeric and dimeric
species of scFv7 showed an off-rate of \( k_{\text{off}} = 3 \times 10^{-3} \text{ s}^{-1} \), which was identical to that of the unfractionated mixture. The on-rate was estimated as \( k_{\text{on}} = 8 \times 10^{4} \text{ M}^{-1} \text{ s}^{-1} \) and the association constant \( K_{\text{aff}} = (k_{\text{on}}/k_{\text{off}}) = 2 \times 10^{6} \text{ M}^{-1} \).

The analyzed scFv7 was also shown to bind to LRP/\( \mu \)MR with high affinity and, to a lesser extent, to LDL-R (Fig. 2A). Affinity constants for human LRP/\( \mu \)MR and bovine LDL-R were therefore also determined. Immobilization of LRP and LDL-R yielded 2100 and 550 RU, respectively. scFv7 at concentrations of 2.5 \( \times \) 10\(^{-7}\) to 2 \( \times \) 10\(^{-6}\) M was tested for binding to the immobilized proteins. scFv7 bound to LRP with an affinity of \( K_{\text{aff}} = 8 \times 10^{7} \text{ M}^{-1} \) and to LDL-R with \( K_{\text{aff}} = 5 \times 10^{7} \text{ M}^{-1} \) (Fig. 3).

As it has been recently shown that RAP also binds strongly to the VLDL receptor of mammalian (58) as well as of avian origin (73), we also analyzed the kinetics of binding of RAP to chicken VLDL receptors. Recombinant RAP fused to glutathione S-transferase (GST-RAP) (15) was injected over a Biacore chip coated with OVR in the presence of 2 \( \times \) 10\(^{-7}\) to 2 \( \times \) 10\(^{-6}\) M, yielding an affinity constant in the same range as scFv7 of 3 \( \times \) 10\(^{8}\) M\(^{-1}\) (data not shown).

Ligand Competition—Competition studies were first carried out using several natural ligands of OVR. Chicken oocyte membrane extract was fractionated on a 4.5–12% gradient SDS-polyacrylamide gel under nonreducing conditions and transferred onto a nitrocellulose membrane. Strips were cut from the membrane and separately incubated with \( 125^{I} \)-scFv7 in the presence of a 1000-fold molar excess of various ligands. VLDL, VTG, GST-RAP, and unlabeled scFv7 strongly competed for binding of \( 125^{I} \)-scFv7 to OVR, whereas excess of the irrelevant scFv D1.3 (31) showed no influence on binding (data not shown). For a more quantitative assay, purified chicken OVR was coated onto ELISA plates and incubated with \( 125^{I} \)-scFv7. Competitors were added at increasing concentrations corresponding to a 2–70-fold molar excess over \( 125^{I} \)-scFv7 (Fig. 4A). Here, a 5-fold molar excess of unlabeled scFv7, VTG, or GST-RAP reduced the binding almost to background. VLDL was much less efficient. Furthermore, rabbit polyclonal antiserum (1000-fold molar excess IgG fraction) to chicken OVR did not compete with binding of the scFv to OVR, suggesting that no (or very low) amount of antibodies binding to the particular epitope recognized by scFv7 were present in the antiserum (not shown). As scFv7 also binds to LRP/\( \mu \)MR of chicken and rat (Fig. 2A), and RAP is known to compete for binding of all known ligands to LRP/\( \mu \)MR (4, 7–9, 15, 16, 59, 60), we wondered whether scFv7 and RAP would compete for binding to human LRP/\( \mu \)MR. As seen in Fig. 4B, GST-RAP completely eliminated binding of \( 125^{I} \)-scFv7 to LRP/\( \mu \)MR at a 7.5-fold molar excess.

Requirement for Ca\(^{2+}\)—Members of the LDL receptor family require Ca\(^{2+}\) ions for ligand binding (9, 40, 57, 61, 62). As scFv7 appeared to be binding at the same sites as the ligands, we checked the influence of Ca\(^{2+}\) ions on the binding of \( 125^{I} \)-scFv7 to chicken OVR and to human LRP/\( \mu \)MR. Oocyte membrane extract was fractionated on a 4.5–12% gradient SDS-polyacrylamide gel, blotted onto a nitrocellulose membrane, and incubated with \( 125^{I} \)-scFv7 in the presence of 20 mM EDTA and EGTA, respectively. Whereas the blot incubated in the presence of 2 mM Ca\(^{2+}\) showed strong binding of the antibody fragment to the receptor, no binding was evident when EDTA or EGTA was added (data not shown). For a more quantitative assay, we used OVR- and LRP/\( \mu \)MR-coated plates, as above, and the binding of \( 125^{I} \)-scFv7 was monitored in the presence of EDTA and EGTA (Fig. 5). Higher concentrations of the chelating agents led to the loss of binding of scFv7 to both OVR (Fig. 5A) and to LRP/\( \mu \)MR (Fig. 5B), and this was reversed by addition of Ca\(^{2+}\) in excess (Fig. 5, C–D).

Competition of \( 35^{S} \)-HRV2 Binding to OVR by scFv7—Human rhinoviruses of the minor receptor group use members of the LDL receptor family for entry into their host cells. It was previously shown that RAP inhibits infection of human fibroblasts deficient in LDL-R synthesis, but expressing LRP/\( \mu \)MR (8). We have recently shown that HRV2 also binds to OVR.\(^{4}\) To study whether scFv7 also competes for HRV binding to OVR, oocyte membrane extract was fractionated on a 4.5–12% gradient SDS-polyacrylamide gel, blotted onto a nitrocellulose membrane, and incubated with 100,000 cpm of \( 35^{S} \)-HRV2 in the presence of 10 \( \mu \)g/mL scFv7 and RAP, respectively. Both proteins were able to completely abolish binding of \( 35^{S} \)-HRV2 to OVR, while a control scFv did not reveal any influence on binding (data not shown). Quantitative competition assays were carried out using ELISA plates coated with OVR. Plates were then incubated with \( 35^{S} \)-HRV2 together with scFv7 or with RAP at the concentrations indicated in Fig. 6. Binding of

\(^{4}\) Gruenberger, M., Wandl, R., Nimpf, J., Hiesberger, T., Schneider, W. J., Kuchler, E., and Blaas, D. (1995) J. Virol., in press.


35S-HRV2 to OVR was reduced to about 10% upon addition of 100 ng/ml RAP and scFv7, respectively, while the control fragment showed hardly any effect on HRV2 binding.

**Inhibition of Viral Infection—**

Human fibroblasts from a patient with familial hypercholesterolemia (FH cells, deficient in LDL-R) were preincubated with scFv7 at various concentrations and infected with either HRV2 or HRV14. Progeny virus was determined by plaque assay and the yields of infectious particles are shown in Fig. 7. From these experiments it becomes clear that scFv7 inhibits viral infection significantly, decreasing the viral yield by more than 3 orders of magnitude at a concentration $\geq 1 \mu$g/ml. The inhibition is specific for the minor group virus HRV2, since infection with HRV14 resulted in essentially the same virus yield regardless of the presence of the antibody fragment (data not shown). Addition of the anti-myc-tag antibody 9E10 decreased the concentration required for inhibition to about 0.2 $\mu$g/ml (data not shown). Presence of their relevant scFvD1.3 used as a control showed no influence on the viral infection.

**DISCUSSION**

The biological functions of the LDL receptor family are important and diverse. For example, the LDL receptor has a key role in cholesterol homeostasis in mammals; mutations disrupting its function leading to severe hypercholesterolemia and premature atherosclerosis in man (63). The LRP/α2MR is probably involved in clearing spent proteases and chylomicron remnants from the circulation (64), and may also have a role in development, as mouse embryos with a homozygous knockout for the LRP/α2MR gene are arrested in various stages of development (59, 65). OVR appears to have a role in reproduction mediating growth of oocytes via uptake of the major yolk precursors VLDL and VTG from coated pits in the plasma membrane (66, 67): mutant “restricted ovulator” hens are sterile.
Inhibition of viral infection of FH cells. Human fibroblasts from a patient with familial hypercholesterolemia (FH cells, deficient in LDL-R, expressing LRPA2-MR) were preincubated with scFv7 at various concentrations and infected with HRV2 at an multiplicity of infection of 10. Progeny virus was determined by plaque assay and the numbers of infectious particles are plotted against the amount of competitor added. The mean values of three experiments are shown, and standard deviations are indicated as error bars.

As the yolk precursor proteins comprise about 50% of the total weight of the egg yolk, the endocytic mechanisms mediated by OVR must be highly efficient.

Antibodies that block the binding of multiple ligands to the LDL receptor family should help in dissecting the roles of these receptors and ligands. Although antisera and monoclonal antibodies have been obtained against several members of the LDL-R family, none have been described that efficiently block the binding of ligands. An exception is IgG-C7, a monoclonal antibody against human LDL-R recognizing the first ligand binding domain of the receptor, which inhibits the binding of LDL or apoE-rich lipoproteins (69). The difficulty in obtaining antibodies with the desired properties by conventional means may be due to the conserved nature of these epitopes of the receptor between different species, or to the presence of ligands in the serum of the animal to be immunized rendering the binding site(s) inaccessible.

We therefore attempted to make blocking antibodies by phage display technology without immunization, and selecting with pure chicken OVR from a large (1.5 x 10⁹) phage library without immunization, and selecting for Binding Site(s) inaccessible.

The protection is even stronger in the presence of the anti-myoc-antibody 9E10 which renders the scFv7 bivalent by binding two molecules via the myc-sequence tag which is COOH terminally fused to the antibody fragment. Based on the dual specificity it is likely that minor group HRVs recognize a structure or charge pattern equally present in LDL-R and LRPA2-MR which might be detectable using antibodies with a broader specificity and improved affinity toward LDL-R. Experiments to produce such antibodies are presently being carried out in our laboratory.

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