Neutralizing human antibodies to varicella-zoster virus (VZV) derived from a VZV patient recombinant antibody library

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Varicella-zoster virus (VZV), the causative agent of chickenpox and herpes zoster, can be life-threatening in prematurely born children and in children with immune defects or who are under immunosuppressive treatment. Therefore agents for passive immunization, such as VZV-specific immunoglobulin preparations (VZIG) derived from convalescent plasma, are crucial in the prophylaxis of VZV infection. This study describes the isolation of human VZV-neutralizing recombinant antibodies. A human single-chain variable fragment (scFv) phage display library was generated from RNA extracted from peripheral blood lymphocytes of a convalescent varicella patient. Specific phage antibodies were selected against VZV-infected human fibroblasts, and eight unique clones were further expressed as soluble scFv in Escherichia coli. They all showed binding characteristics to varicella antigens with affinities in the $K_D$ range $0.1 - 0.2 \mu M$. Two of the scFv antibodies, VZV4 and VZV5, showed dose-dependent in vitro neutralization of VZV. VZV39 also showed a neutralizing effect as scFv, an effect that was increased 4000-fold by conversion into IgG and was further increased by the addition of complement. This is possibly the first time that monovalent scFv antibodies have been shown to neutralize VZV in vitro. This finding will have an impact on the production of new prophylactic antibodies, as such antibody fragments can be cost-effectively produced in E. coli. The antibodies isolated bind both complement-dependent and -independent epitopes for neutralization, thus they may prove useful tools for the study of VZV virulence mechanisms.

INTRODUCTION

Varicella-zoster virus (VZV), human herpesvirus 3, is the causative agent of chickenpox and herpes zoster. VZV is spread by the respiratory route and disseminates to lymph nodes and further via lymphocytes back to the skin, resulting in the rash of chickenpox. VZV also infects the dorsal root ganglia where it causes lifelong latency. The virus reactivates in association with declining T-cell immunity, causing herpes zoster or shingles. Although VZV is a relatively harmless infection to most individuals, it may be life-threatening for prematurely born infants, children with immune defects or children under immunosuppressive treatment, e.g. because of cancer therapy. Thus, hospitalized individuals at risk are subject to passive immunization by VZV-specific immunoglobulin preparation (VZIG). This immunoglobulin preparation is derived from human pooled sera or plasma of VZV convalescent donors. Although effective, there are obstacles associated with such intravenous immunoglobulin preparations. The supply is limited, the volumes administrated are large and, as with any human blood product, it may carry a potential risk associated with contaminating infections. Monoclonal antibody preparations overcome many of the problems inherited in pooled human sera, as the concentration of virus-specific antibodies is several orders of magnitude higher than in pooled sera, and the transmission of disadvantageous pathogenic agents is totally avoided. However, the production cost of human monoclonal antibodies is still a major obstacle.

Only a few human monoclonal antibodies against VZV have been generated to date (Foung et al., 1985; Sugano et al. 1987; Lloyd-Evans & Gilmour, 2000). The generation of human hybridomas is often associated with instability of the cell line. Thus, alternative methods for isolating...
human antibodies are in great demand. With the introduction of phage display technology the generation of antibody libraries is now being performed routinely, and they have been used successfully for the isolation of antibodies to virtually any antigen (Griffiths et al., 1994; Vaughan et al., 1996; Sheets et al., 1998; de Haard et al., 1999; Little et al., 1999; Knappik et al., 2000; Sblattero & Bradbury, 2000; Soderlind et al., 2000). However, whereas the isolation of specific antibodies against a pathogen is routine, it is more difficult to isolate functional antibodies (the neutralizing antibodies). As long as the antibody can be detected in the serum of a patient, it can also be isolated from the corresponding library from this donor (Williamson et al., 1993). Such patient-derived antibodies have developed to fight infections and thus are probably the best source for functional anti-infective antibodies.

VZV is an enveloped virus expressing seven glycoproteins in the membrane, of which glycoprotein E (gE) is highly immunogenic. However, the VZV-specific antibody with the highest documented neutralization titre is directed against glycoprotein H (gH) (Grose, 1990). One antibody, a murine monoclonal antibody against gH, mAb 206, is shown to neutralize VZV in the absence of human complement (Montalvo & Grose, 1986). Recently, it was reported that humanized fragments of this antibody neutralized VZV, although monomer fragments did not (Drew et al., 2001).

We have utilized recombinant antibody technology to isolate and select VZV-neutralizing human antibodies from an antibody phage display library derived from a varicella patient. The isolated clones show a high neutralization effect both as monomer single-chain variable fragments (scFv) and as intact immunoglobulins. This is, to our knowledge, the first report of VZV neutralization of human monomeric scFv fragments. These antibodies may also serve as agents for the prophylaxis and post-infection prophylaxis of VZV infection.

**METHODS**

**Antigen preparation**

**Cloning and expression of recombinant VZV gE.** DNA sequence information of gE was obtained from EMBL: HEVZVXX, accession no. X04370 (Davison & Scott, 1986). Oligonucleotides for the amplification of gE extracellular parts were designed: 1, 5’-AGAG-AGCACGTGGTATACGAACTCGGTGAGA-3’; 2, 5’-AGAGAG-GCGGCCGCCCTGCTGAAAGTGTGACGT-3’.

The PCR product includes restriction-enzyme cleavage sites for PvuII and NotI (underlined) in oligonucleotides 1 and 2, respectively. The PCR product was digested with PvuII and NotI enzymes and cloned into a version of pHOG dummy (Stacy et al., 2003) in which the C-terminal myc and his polypeptide tags are removed and a FLAG tag is inserted N-terminal to the gE protein. The construct was sequenced and verified as the extracellular part of gE. Recombinant FLAG-gE was expressed in E. coli. The denaturation and renaturation of gE inclusion bodies produced soluble recombinant gE. The recombinant gE was verified as structurally functional by positive binding of gE-specific monoclonal antibodies (data not shown).

**Generation of VZV cell lysate and control antigen.** Early passage laboratory strain RG was used for infection of human embryonic fibroblasts (HE cells). HE cells did not undergo more than 17 passages before use. Then 0.5–1.5 ml virus incubated/absorbed the cells at 1–1.5 h at room temperature. Eagle’s minimal essential medium supplemented with penicillin and streptomycin, 10% fetal calf serum and 1 ml 5% NaHCO3 per 100 ml medium was added and further incubated at 37 ℃ overnight. The medium was changed after 24 h, and cells were harvested after 52 h. Cells were sonicated for 15 s and centrifuged at 1000 r.p.m. Non-infected cells were treated in an identical way for the generation of control antigen.

**Isolation of lymphocytes and serum from the patient.** Blood was drawn on days 5 and 11 after appearance of the first vesicular lesion on an adult male with primary varicella infection. Blood (10 ml) was spun at 4000 r.p.m. (2969 g) for 15 min and the serum was frozen at −20 ℃. Peripheral blood lymphocytes were isolated by lymphoprep separation (Boyum, 1968). The mononuclear cells were washed twice in PBS. Cells were counted and frozen in batches of 5 x 10^7 cells.

**ELISA of patient serum.** Microtitre plates were coated with 4 μg VZV cell lysate and control antigen ml⁻¹. All sera were diluted 1:50 in water and 100 μl of each diluted serum was added to both the VZV cell lysate and control antigen-coated microtitre wells.

The negative serum was from a person with no visible signs of ever having had a VZV infection. The positive control is serum from a person who had a varicella infection many years ago (low-positive), mAb7.88, a mouse anti-VZV monoclonal antibody (Norwegian Institute of Public Health) was diluted 1:1000 before use.

**RNA isolation and cDNA synthesis.** Cells (2 x 10^7) were washed once in ice-cold PBS, and the cell pellet was lysed. Between 5 and 25 μg total RNA was isolated by Strataprep Total RNA miniprep kit, as described in the manufacturer’s protocol (Strategene). Donor RNA was used for first strand cDNA synthesis by way of random hexamer oligonucleotides according to the protocol of SuperScript RNase H⁻ reverse transcriptase (Gibco-BRL). Briefly, 5 μg RNA, 0.5 mM dNTPs (Fermentas) and 500 ng random hexamers (Promega) were incubated at 65 ℃ for 5 min, followed by rapid cooling to 4 ℃. Then, 5 μl reverse transcriptase reaction buffer (Gibco-BRL), 10 mM DTT and 2 U RNasin μl⁻¹ (Promega) were added and incubated at 25 ℃ for 2 min. During incubation, 200 U SuperScript II reverse transcriptase (Gibco-BRL) was added. After 10 min, the reaction was incubated at 42 ℃ for 1 h. The reverse transcriptase was inactivated at 70 ℃.

**Variable (V) gene PCR amplification.** Oligonucleotides used for the primary amplification of V genes are described by Sblattero & Bradbury (1998). A secondary set of oligonucleotides was designed, including restriction enzyme sites in the 5’ ends corresponding to the VH and VL cloning sites (see below). The PCR reactions were thus performed in two steps. First, 125 ng cDNA was used as template for the amplification of V genes. A total of nine IgG variable heavy-chain (VH), 20 variable light-chain kappa (Vκ) and nine variable light-chain lambda (Vλ) reactions were set up with V-gene-specific oligonucleotide sets. The reactions were run at annealing temperatures of 55, 58 or 61 ℃ (depending on the primer pair) for 30 cycles with 2 U Vent DNA polymerase (New England Biolabs) and 20 pmol of each primer pair per reaction.

One microlitre of each primary PCR reaction (~ 100 ng) was used as template for the secondary PCR reactions. A total of 45 VH, nine Vκ and 20 Vλ PCR reactions were run for 30 cycles at an annealing temperature of 58 ℃. All the oligonucleotides used in the secondary PCR introduce restriction enzyme sites: NotI and HindIII for all VH genes, and MluI and NotI for all Vκ and Vλ genes.
Cloning of V genes into phagemid pSEX81. A modified pSEX phagemid (G. Å. Låset, Affitech AS, personal communication) was digested with MluI and NotI and ligated with Vs and Vz pools and electroporated into XL-1 Blue cells. The two light-chain libraries (Vs and Vz) showed between 3 × 10⁶ and 5 × 10⁶ clones. The bacterial colonies were scraped from agar plates and a total plasmid preparation from each library was isolated by DNA miniprep (Qiagen).

The light-chain plasmid libraries (pSEX VL) were digested with NotI overnight at 37 °C. Linearized plasmid was isolated from agarose gels and purified by gel extraction kit (Qiagen) followed by secondary digestion with HindIII overnight. The digestion reaction was incubated with calf intestinal phosphatase (New England Biolabs) before the double Ncol/HindIII-digested plasmid was isolated from agarose gel and purified by gel-extraction kit (Qiagen).

The Ncol and HindIII-digested pSEX VL plasmids were ligated with seven variable heavy chain pools and electroporated into XL-1 Blue cells. Colonies were grown on agar plates with ampicillin (100 μg ml⁻¹) and 100 mM glucose.

Packaging of phage libraries. The plates with pSEX library-transformed E. coli were scraped. 2 × YT (50 ml; Difco) with 100 μg ampicillin ml⁻¹, 30 μg tetracycline ml⁻¹, 100 mM glucose (2 × YT-ATG) was inoculated to OD₆₅₀ value of 0.025. The culture was incubated at 37 °C for 4 h. At OD₆₅₀ value of 0.1, helper phage M13KO7 was added at an m.o.i. of 8. The infection continued at 37 °C for 30 min at 80 r.p.m. and for 30 min at 260 r.p.m. The culture was centrifuged at 4000 r.p.m. (9985 g) for 30 min. The phage pellet was dissolved in 1 ml TE buffer (pH 7.4) and transferred to microcentrifuge tubes for the last centrifugation at 14 000 r.p.m. for 10 min to remove insoluble material.

Panning conditions. In both rounds of selection the library was pre-incubated with control antigen. Unbound phages were subjected to further incubation with VZV cell lysate antigen. Antigen was immobilized in immunotubes (NUNC) in bicarbonate buffer pH 9.6 at concentration of 300 μg ml⁻¹ in the first round and 30 μg ml⁻¹ in the second round. After 10 times washes with PBS/0.05% Tween 20 followed by 10 times washes with PBS, which was increased to 20 times washes in the second selection round, the bound phages were eluted with 500 μl 37% triethylamine followed by neutralization with 500 μl Tris/HCl pH 5.5. The eluted phages were allowed to infect XL-1 Blue at OD₆₅₀ value of 0.4. The infected cells were plated on LB-ATG plates and incubated at 30 °C overnight. After the first round of selection, colonies were scraped and handled as described in the previous section. From both rounds, single colonies were picked and monoclonal phages isolated and assayed by ELISA.

V-gene analysis. Sequencing was performed at GATC GmbH, Constance, Germany. All sequenced V genes were analysed by DNAPLOT at the V-base (www.mrc-cpe.cam.ac.uk). All amino acid sequence alignments were performed using CLUSTAL W 1.8 on the BCM-search launcher (http://searchlauncher.bcm.tmc.edu).

Expression and purification of soluble scFv. The scFv genes from round two were collectively cloned into the pHOG expression vector as Ncol/NotI-digested inserts (Stacy et al., 2003) and transformed into XL-1 Blue. One hundred clones were picked and expressed in 96 deep-well plates. The plates were centrifuged and supernatants subjected to ELISA as described below. ELISA-positive colonies were picked from the master-stock plates and grown overnight at 37 °C in LB-ATG. The overnight culture from each positive clone was further inoculated into LB-ATG medium to OD₆₅₀ value of 0.1. The cultures were grown to OD₆₅₀ value of 0.8. Induction was performed by changing medium to LB with 100 μg ampicillin ml⁻¹ and 100 μM IPTG and incubated overnight at 30 °C. scFv in the supernatant and periplasmic fractions were subjected to purification by metal-chelating chromatography by Ni²⁺ chelating sepharose and eluted by 250 mM imidazole. The purified scFv were quantified by SDS-PAGE, ELISA and OD₂₈₀ measurements.

Cloning and expression of intact IgG. The V genes from selected clones were amplified by oligonucleotides specific for V genes with extensions including restriction–enzyme cleavage sites for cloning into the eukaryotic expression vectors pLNOH2 and pLNOI, as described (Norderhaug et al., 1997). The plasmids were co-transfected into N50 cells by electroporation. For the selection of resistant clones, 600 μg G418 ml⁻¹ was added to transfected cells. Emerging G418-resistant colonies were analysed for production of soluble IgG by ELISA. Positive clones were subjected to limiting dilution followed by ELISA, the highest-producing clones were expanded, and the supernatant harvested.

ELISA. Ninety-six-well microtitre plates (Maxisorp; NUNC) were coated with either 4 μg VZV cell lysate and control antigen ml⁻¹, or with 20 μg recombinant gE and BSA ml⁻¹ as control overnight at 4 °C. The wells were blocked with 4% skimmed milk before dilutions of scFv or IgG were added and incubated for 1 h at room temperature. Plates were washed with PBS/Tween followed by incubation of anti-myel antibody (1:5000) for scFv detection or anti-human Fc antibody (1:10000) for IgG detection for 1 h at room temperature. After washing in PBS/Tween, HRP-labelled anti-mouse IgG (1:20000) was added and incubated for 1 h at room temperature. The plates were washed in PBS/Tween and signal developed by adding ABTS (Calbiochem). The plates were read at OD₄₀₅ after 20 min.

Surface plasmon resonance (SPR) analysis of anti-gE scFv. The CM5 dextran sensor chip was activated with a 30 μl (6 min) injection of 0.2 M N-ethyl-N²-(3-dimethylaminopropyl)-carbodiimide + 0.05 M N-hydroxysuccinimide (EDC/NHS) followed by a 30 μl injection of 1 M ethanolamine hydrochloride to deactivate excess NHS esters. The recombinant gE protein was amine-coupled at a concentration of 0.75 mg ml⁻¹. The scFv-binding analyses were performed on a BiacoreX (Biacore AB) at 25 °C with a flow rate of 50 μl min⁻¹.

VZV-neutralization assay. A sensitive VZV micro-immunoplaque was used to analyse isolated scFv fragments and IgG molecules for the ability to neutralize VZV. Briefly, VZV cell-free stock (2–5 × 10⁶ p.f.u. ml⁻¹) in optimal dilution were mixed with dilutions of scFv or IgG of the various anti-VZV clones, and incubated at room temperature for 1 h. The mixtures were then added to confluent monolayers of HE cells and incubated for another hour at room temperature before further incubation in a 5% CO₂ cabinet at 34.5 °C. After incubation, the cell monolayers were fixed using a mixture of ice-cold (−20 °C) acetone–methylene (70:30) containing 0.5% Triton X-100. The VZV plaques were visualized using a VZV-specific mouse monoclonal antibody (mAb gp7,88) and a peroxidase-conjugated rabbit anti-mouse IgG polyclonal serum (P0260; Dako). In this study, a human serum selected from a VZV- and HSV-negative blood donor was used as a source of complement (80 IU ml⁻¹). Controls used in various assays were an scFV clone of irrelevant specificity (anti-flunitrazepam), the RN donor serum (convalescent varicella patient), a VZV- and HSV-negative human serum, and an IgG1 human monoclonal antibody of irrelevant specificity.
specificity (anti-6-mono-acetylmorphine). As a complement control
the same serum as above was used, but had been inactivated by
heating at 56 °C for 30 min.

RESULTS AND DISCUSSION

Generation of library and selection of antibodies

We have utilized the antibody repertoire generated in
an individual who had acquired varicella. The patient’s
lymphocytes were isolated on day 11 after an outbreak,
the time point corresponding to high VZV antibody serum
titre (data not shown). The two-step cloning procedure
from RT-PCR V genes first generated a VL repertoire.
Subsequently, the VH repertoire was cloned into the VL
library to constitute a total library of $5 \times 10^7$ different
antibody clones. The phage display antibody library,
containing 10 000 copies of each clone, was subjected to
selection on VZV-infected human fibroblasts. To reduce
the amount of unspecific antibodies and antibodies bind-
ing to human fibroblasts (self-antigens), the library was
pre-incubated with human fibroblasts before each round
of selection against VZV-infected cells was performed.
After the first round of selection, 2 % VZV phage-specific
antibodies were disclosed. The percentage of phage-specific
antibodies increased to 35 % after the second round of
selection (Fig. 1). We decided to stop selection at the second
round to reduce the bias that is otherwise known to occur
after several rounds of phage display library selections.

Cloning and expression of the antibodies

As the selected library contained as much as 35 % VZV-
specific antibodies, we decided to clone all V genes
collectively from the second round of selection into the
scFv expression plasmid pHOG dummy (Stacy et al., 2003).

Single colonies were randomly picked and expressed as
soluble scFv. One hundred expressed clones were screened
for binding to VZV cell lysate, control lysate, recombinant
gE or BSA (data not shown). From this screening we found
20 positive anti-VZV antibodies, which were identified as
eight unique clones by DNA sequencing. The deduced
amino acid sequences are shown in Fig. 2.

After purification, the SDS-PAGE revealed scFv with
molecular masses ($M_r$) in the range 27–30 kDa (Fig. 3).
Although the scFvs are similar, the differences in $M_r$ are
due to the different lengths of the CDR3 peptide regions
of the VH. These peptide stretches are the most diverse
regions in the antibody structure. Analysis of the DNA
sequences revealed typical V-gene usage, with predomi-
nantly VH3 family for the heavy-chain and both V¿ and
Vk light-chain families (Table 1). It has been shown (G. Å.
Løset, Affitech AS, personal communication) that the
oligonucleotide sets used to amplify the V genes indeed
pick up all known V-gene families. Thus, the result of this
narrow V-gene family usage is because of positive selection
of the repertoire that has been generated by the donor to
bind VZV antigens, not because of a bias in the PCR
amplification procedures.

Antibody specificity and affinity

Three clones, VZV18, VZV38 and VZV39, showed specifi-
city to varicella gE, whereas the other five clones bound yet
undefined varicella antigens in the VZV lysate. For technical
reasons, we carried out SPR analysis only of the gE-specific
antibodies, which revealed affinities in the submicromolar
range (Fig. 4; Table 1). These binding measurements are
probably performed by monomer scFvs and thus constitute
medium antibody affinity. We must emphasize that none
of the selected antibodies gave high ELISA values when
assayed on their respective antigens, thus the antibodies
selected in this project are probably low- to medium-affinity
binders. This result can be explained partly by the selection
method. We deliberately did not select the antibodies
under stringent conditions as we wanted to isolate as many

![Fig. 1. ELISA assay of phage displayed antibodies. ELISA signals from randomly picked phages are shown after selection
rounds one and two of library selection against VZV cell lysate. Each phage is assayed against VZV cell lysate (VZV, grey
bars) and against non-infected cells (VZV-ctr, black bars). For details see Methods.](image-url)
and as diverse antibodies as possible. Performing selection under highly stringent conditions we might get high-affinity binders, but not necessarily the most functional antibodies with respect to neutralization. It is, however, important to address the question why we did not get any high-affinity binders at all. We can only speculate that this donor did not generate such antibodies. This seems reasonable as the B lymphocytes were taken from a person with primary VZV infection only 5–11 days after clinical symptoms. At this time it is conceivable that the immune system had not had sufficient time to establish high-affinity antibodies.

**VZV neutralization by scFv antibodies**

All scFvs isolated were tested in neutralization of VZV in an immunoplaque reduction assay. Six of eight clones showed a neutralizing effect (Fig. 5). Clones VZV2, VZV18 and VZV16 only inhibited VZV infection by 10–20%. Clone VZV39 showed 40% inhibition, whereas clones VZV4 and VZV5 were close to 100% neutralization at a concentration of 10 μM (Fig. 5). In repeated assays, VZV4 and VZV5 performed neutralization in a dose-dependent manner (Fig. 6). It is always difficult to predict how scFv appear in solution under different assay conditions. It has been reported that scFv might appear as monomers, dimers or trimers, or even tetramers (Brekke & Sandlie, 2003). In the neutralization assay we have not proven a strictly monomeric appearance of scFv. However, all scFv in our expression system contain an identical peptide-linker region (Fig. 3), which has been shown to promote monomer...
structures (Le Gall et al., 2004). Thus, we assume that at least a high proportion appear as monomers in the neutralization assay. This is, to our knowledge, the first time VZV-neutralizing monomer antibody fragments have been described. The two most potent scFv antibodies, VZV4 and VZV5, showed IC50 values of 5 and 0.23 μM, respectively (Table 1).

VZV neutralization by IgG antibodies

The V genes of clones VZV4, VZV5 and VZV39 were cloned into eukaryotic expression vectors and expressed in NS0 cells as intact human IgG1/λ antibodies. As intact IgG mediate effector functions, we could study the neutralizing capacity of these antibodies with or without complement. For clone VZV39, increased effect was observed by the addition of complement, whereas for clones VZV4 and VZV5 only marginal effects were detected (Fig. 7). For the donor serum RN11 increased effect with complement was observed. In these assays the complement sources were

Table 1. Summary of anti-VZV antibody characteristics

Antibody specificities to either glycoprotein E or unknown antigens in the viral lysate. V-gene families of the variable heavy chain (VH), variable λ light chain (VL) or variable κ light chain (Vk). The affinities measured by SPR analysis are shown as dissociation constants (Kd). Also shown are IC50 values for clones VZV4, VZV5 and VZV39.

<table>
<thead>
<tr>
<th>Clone</th>
<th>Specificity</th>
<th>VH family</th>
<th>VL family</th>
<th>Kd (M)</th>
<th>IC50 scFv (μM)</th>
<th>IC50 IgG (nM)</th>
<th>IC50 IgG+C+ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VZV4</td>
<td>Lysate</td>
<td>VH3</td>
<td>Vl2</td>
<td>–</td>
<td>5</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>VZV5</td>
<td>Lysate</td>
<td>VH3</td>
<td>Vκλ</td>
<td>–</td>
<td>0.23</td>
<td>1.3</td>
<td>1</td>
</tr>
<tr>
<td>VZV39</td>
<td>gE</td>
<td>VH2</td>
<td>Vκ3</td>
<td>1.6 × 10^-7</td>
<td>11*</td>
<td>5</td>
<td>0.3</td>
</tr>
<tr>
<td>VZV18</td>
<td>gE</td>
<td>VH3</td>
<td>VκIII</td>
<td>1.0 × 10^-7</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>VZV38</td>
<td>gE</td>
<td>VH1</td>
<td>VκII</td>
<td>1.8 × 10^-7</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>VZV11</td>
<td>Lysate</td>
<td>VH3</td>
<td>Vκ1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>VZV16</td>
<td>Lysate</td>
<td>VH3</td>
<td>Vκ2</td>
<td>–</td>
<td>–</td>
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* Approximation of IC50 value from scFv data (not shown).
human serum, and inactivated serum from the same donor was used as complement control. Thus, the increased effect of VZV39-IgG upon complement addition reflects complement activation, not merely a multimeric effect by the binding of antibodies to the C1 complex.

The observation that VZV4 and VZV5 were not affected by complement, together with the fact that they neutralized VZV as scFv, underscores the finding that VZV4 and VZV5 bind VZV epitopes that are not complement-dependent. It has been stated that gH contains neutralizing epitopes that are not complement-dependent (Montalvo & Grose, 1986). Thus, VZV4 and VZV5 may indeed bind to gH.

The functional concentration of antibodies is composed of a variety of elements such as affinity, specificity, valency, kinetics and external factors (Brekke & Sandlie, 2003). In this study, we observed an 180-fold increase in IC$_{50}$ from the scFv format to IgG format for VZV5, and over 2000-fold for VZV39 (Table 1), indicating a beneficial effect of bivalency and thus avidity. As the affinity of VZV39 is in the low micromolar area, we assume that the IC$_{50}$ value can be reduced considerably if we are able to increase the antibody affinity. Methods for in vitro enhancement of antibody affinities are based on the introduction of mutations in the V genes, or introducing new variable light-chain repertoire together with the original variable heavy chain thus creating a small library repertoire of slightly altered antibodies. The subsequent small library is subjected to high-stringency selection. Thus, only antibodies with higher affinity than the mother clone will be selected (Brekke & Sandlie, 2003). By the induction of complement, VZV39-IgG increased potency 16-fold from 5 to 0.3 nM (Table 1). Compared with VZV4-IgG and VZV5-IgG, VZV39-IgG was about 100 and three times more potent, respectively. By genetic engineering of the IgG Fc-region, antibodies with higher complement-activation potential can be generated (Michaelsen et al., 1990; Brekke et al., 1993). In this study, we have developed potent antibodies that can gain even higher functionality by the employment of genetic engineering of both the antibody affinity and effector functions.

Concluding remarks

The use of antibodies derived from human antibody libraries may prove to be a very successful method to develop neutralizing antibodies against certain human viruses. The isolation of one in vitro neutralizing human antibody against the S1 protein of SARS coronavirus derived from a phage display library has been described recently (Sui et al., 2004). It may also prove that human antibodies derived from human donors, as described here, will help the effort to produce better medicines for passive immunity, an important aspect of the increasing resistance to both antibiotics and anti-viral drugs.

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