Comparison of Two Single-Chain Antibodies That Neutralize Canine Parvovirus: Analysis of an Antibody-Combining Site and Mechanisms of Neutralization

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Received January 7, 2000; accepted February 2, 2000

We cloned the heavy- and light-chain variable domains of two monoclonal antibodies that recognize each of the two major neutralizing antigenic sites of the canine parvovirus (CPV) capsid. After expression in Escherichia coli as single-chain variable domains (scFv) with glycine-serine linker sequences, both scFv bound CPV capsids with the same specificity as the intact IgG, but with 10- to 20-fold lower avidity. Both scFvs neutralized CPV infectivity with efficiency similar to that of the IgG. Although both IgGs inhibited hemagglutination by CPV, only one scFv was inhibiting. The binding of one of the antibodies has previously been analyzed by cryoelectron microscopic reconstruction and the epitope-binding residues predicted. Mutagenesis of predicted contact residues in three heavy-chain complementarity-determining regions (CDR) showed that mutants of CDR1 or CDR3 reduced the binding of the scFv by about 10-fold compared with the wild-type scFv, while no effect was seen for one mutant of CDR2. The levels of neutralization of CPV and of hemagglutination inhibition by the scFv mutants were proportional to their reduction in binding affinity compared with the wild type. Neither scFv blocked virus binding to host cells, but they both caused aggregation of the capsids and appeared to affect the process of infection after virus uptake into the cells. © 2000 Academic Press

INTRODUCTION

Antibodies are a major host defense against many viruses in vertebrates, and the mechanisms of interaction between the antibodies and viral antigens are now becoming understood. The interactions involve antibody binding to the viral structures in a number of different ways, including binding to flexible loops (Logan et al., 1993; Verdaguer et al., 1999) or to structures that are determined by the folded polypeptide (Colman et al., 1987; Malby et al., 1993; Bizebard et al., 1995; Che et al., 1998; Malby et al., 1998). The mechanisms of virus neutralization can include virus aggregation, masking of receptor attachment sites, steric inhibition of receptor binding, or stabilization of the capsid to inhibit virus uncoating (Outlaw and Dimmock, 1991; Smith, 1993; Verdaguer et al., 1997; Fleury et al., 1999; Sattentau et al., 1999; Smith and Baker, 1999).

The specific interactions between antibodies and antigens involve multiple contacts between the antibody-combining site and the epitope (Davies et al., 1990). When examined at high resolution, changes in the structures of both the viral epitope and the antibody-combining site that allow the effective binding are seen (Colman et al., 1987; Bizebard et al., 1995; Smith and Baker, 1999). Antigenic variants of many viruses can arise through single-sequence substitutions within antibody-combining sites, and those viruses often show reduced or no neutralization by the antibodies (Harris et al., 1977; Wiley et al., 1981; Sobrino et al., 1989; Strassheim et al., 1994; Fleury et al., 1998). That variation may be constrained by the ability of the virus to accommodate structural variation, and viruses differ in their ability to naturally change their antigenic properties.

The parvovirus capsid is 26 nm in diameter, is made up of 60 copies of a combination of VP1 and VP2 proteins, and has \( T = 1 \) icosahedral symmetry (Tsao et al., 1991; Xie and Chapman, 1996; Weichert et al., 1998). Capsid structures have been determined for canine parvovirus (CPV), for the antigenically variant feline panleukopenia virus (FPV), and for a CPV mutant with VP2 residue 300 Ala changed to Asp (Tsao et al., 1991; Agbandje et al., 1993; Wu and Rossmann, 1993; Llamas-Saiz et al., 1996). The CPV antigenic structure has also been examined using peptide analysis and by monoclonal antibody (MAb) analysis of antigenically variant viruses (Lopez de Turiso et al., 1991; Langeveld et al., 1993; Strassheim et al., 1994). The variant virus analysis showed two major neutralizing antigenic sites (termed sites A and B) that were recognized by many MAbs, and natural variants of both sites A and B have been selected in nature (Parrish et al., 1985, 1991; Strassheim et al., 1994).

The binding of one MAb (MAb 8, also referred to as A3B10) to site B was examined using cryoelectron microscopy, mutations within the B antigenic site have...
been defined, and the structure of a CPV mutant that did not bind MAb 8 has been solved (Wikoff et al., 1994; Llamas-Saiz et al., 1996; Parker and Parrish, 1997). The MAb 8 Fab protrudes from the virus with the long axis in a radial direction but leaning away from the nearest twofold axis, indicating that bivalent binding of one IgG to a single capsid would not occur. The two variable domains straddle a ridge on the CPV surface composed of residues 296 to 303, within which two escape mutations have been located (VP2 residue 300 Ala to Asp and residue 302 Asn to Asp) (Strassheim et al., 1994; Llamas-Saiz et al., 1996).

Antibodies and antigens interact through extensive contacts between the complementarity-determining regions (CDRs) of the light and heavy chains of the antibodies and the antigen. The contacts between the heavy-chain CDRs and antigen generally are more extensive, and V\textsubscript{H} CDR3 is often particularly important in determining the antigen specificity (Branden and Poljak, 1995; Padlan et al., 1995). For MAb 8 the V\textsubscript{H} CDR1 and CDR3 appeared to make contacts with the CPV surface, through Asp31 and Tyr32 in CDR1, Asn52 in CDR2, and Gly100 and Tyr101 in CDR3 (Wikoff et al., 1994).

Bacterial expression of an antibody variable domain allows for production of a single-chain variable fragment (scFv), and those expressed domains provide useful tools for studying antibody structure and functions (Skerra et al., 1991; Raag and Whitlow, 1995).

To define the mechanisms of antibody specificity and to analyze the binding and neutralization of CPV, we cloned the variable domains of MAbs which recognize the CPV A (MAb 14) or B (MAb 8) antigenic sites. These variable domains were expressed in bacteria as scFv, and they had binding properties similar to those of the MAb from which they were derived. Both scFvs efficiently neutralized CPV infectivity, but only one of them inhibited viral hemagglutination (HA). Mutagenesis of heavy-chain CDRs of one scFv confirmed that residues important for binding the B antigenic site were within both the heavy-chain CDR1 and the CDR3. Mechanisms of neutralization included aggregation of the capsids and inhibition of infection after binding and entry into cells.

RESULTS

Variable region cloning, sequencing, and expression

The variable domain cDNA sequences of the heavy- (V\textsubscript{H}) and light-chain (V\textsubscript{L}) genes of MAb 8 and MAb 14 were amplified from mRNA by RT-PCR, cloned into plasmids, and sequenced. By use of the Sequence Subgrouping program in the Kabat database, the V\textsubscript{H} of MAb 8 was grouped within the miscellaneous subgroup, and the V\textsubscript{H} of MAb 14 was within subgroup IIB. Both MAbs contained the \kappa light chain: the V\textsubscript{L} of MAb 8 was in subgroup VI; the V\textsubscript{L} of MAb 14 was in subgroup I. The CDR assignment within the MAb sequences was based on the Kabat numbering scheme (Fig. 1). The variable domain sequences were recloned into the expression vector pET22b(+) by overlapping extension PCR, adding the pel B signal sequence and a six-histidine C-terminal tag (Fig. 2). Clones of both scFvs were obtained with either two or three repeats of the Gly-Gly-Gly-Gly-Ser linker sequence. Both scFvs containing the three-repeat linker were expressed in bacteria, and the molecular weights of those on the SDS–PAGE appeared close to the predicted sizes (results not shown). When versions of the scFvs that contained two copies of the G\textsubscript{S} linker sequence were tested, that derived from the MAb 8 clone still bound to CPV, whereas the scFv from MAb 14 did not bind to any detectable degree (results not shown). Those results are similar to those reported for other antibodies in which the ability of the V\textsubscript{H} and V\textsubscript{L} domains to associate correctly was determined by the length of the linker sequence (Malby et al., 1993; Raag and Whitlow, 1995).

The scFvs bound CPV with the same specificity as the original MAb and neutralized CPV infectivity

Purified bacterially expressed scFvs were tested for binding in ELISA to CPV or FPV capsids, or to denatured CPV capsids, and compared with the intact MAbs. Each scFv bound to CPV with the same specificity as the original MAb, as scFv-8 bound efficiently to both CPV and FPV but not to the denatured capsids, while scFv-14 bound to CPV, but not to FPV or to denatured CPV capsids (Fig. 3). However, the binding efficiencies of both scFvs were 10- to 20-fold lower than that of the original MAb (Figs. 3A and 3B).

The scFvs were tested for neutralization of CPV infectivity on A72 cells and NLFK cells by TCID50 assay. The MAb and scFv all showed similar efficiencies of neutralization of CPV when tested on NLFK cells (Fig. 4A) or on A72 cells (results not shown).

scFv-8, but not scFv-14, inhibited viral HA

MAb 8 and MAb 14 both inhibit viral HA (Table 1) (Strassheim et al., 1994). scFv-8 inhibited HA with a 10-fold reduced efficiency compared with MAb 8 (Table 1). However, scFv-14 did not show any HA inhibition even at 400 nM, while MAb 14 inhibited 4 HA units of CPV at 0.125 nM (Table 1). When anti-Fab antibody was added to the reaction, the efficiency of HA inhibition by both scFvs increased, as scFv-8 showed HA inhibition at 0.0625 nM and scFv-14 was active at 10 nM.

Mutations in V\textsubscript{H} CDRs of scFv-8 reduced the binding affinity, neutralization efficiency, and HA inhibition

Changing one or two predicted contact residues in scFv-8 CDR H1, H2, or H3 gave scFv that still bound virus; however, when tested in competition ELISA the H1 and
H3 mutants bound to CPV with about 10-fold lower affinity than wild type, while the H2 mutant binding was the same as wild type (Fig. 5B). The scFv-8 with a combination of the H1 and H3 mutations bound about 2-fold less efficiently than either of those single mutants, while the H1 or H3 mutation along with the H2 mutation showed no additional effect on binding compared with the H1 or H3 mutation alone (Fig. 5B).

The three mutants which showed reduced binding affinity were tested for the neutralization efficiency and HA inhibition activity. The lower affinity scFv mutants showed a two- to fourfold lower efficiency of neutralization when tested on both NLFK cells (Fig. 4B) and on A72 cells (results not shown). The scFv-8 mutants also showed reduced HA inhibition activity compared with wild type (Table 1).

Both scFvs and MAbs aggregated virus

To investigate the neutralization mechanisms, the scFvs or the antiviral antibodies were incubated with 125I-labeled capsids and the mixtures were then centrifuged in sucrose gradients. The capsids without either scFvs or antibodies formed a well-defined peak one-third of the way down the gradient; however, adding polyclonal rabbit anti-CPV antiserum capsids caused 97.7% of the virus to be pelleted. Both scFv-8 and scFv-14 aggregated the capsids, and the aggregates increased with increased amounts of scFvs (Fig. 6). MAb 8 and MAb 14 aggregated the virus, but aggregation occurred most efficiently at a 1:0.2 ratio of capsid VP2 to monomeric MAb binding site, with less aggregation at either fivefold higher or lower ratios of IgG (Fig. 6).

scFvs or antiviral antibodies did not block virus binding and entry to cells

In initial tests, virus was incubated with cells at 4, 20, and 37°C. Although binding to NLFK cells was similar at those three temperatures, binding to A72 cells was barely detectable at 4°C and was greatest at 37°C (results not shown). At 4°C with NLFK cells or at 37°C with A72 cells the virus along with 1:5, 1:1, or 1:0.2 ratios of capsid VP2 to scFv or IgG combining site showed no decrease in cell binding, and in most cases showed a relative increase (Fig. 7). When cells were incubated with virus in the presence of the antibodies, the virus appeared as relatively large bright spots, most likely resulting from aggregation of the virus. In the presence

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of scFvs the virus within the cells also appeared to show some level of aggregation, although the complexes appeared smaller (Fig. 8).

**DISCUSSION**

Here we examined the mechanisms of binding and neutralization of CPV by scFv derived from two well-characterized anticapsid MAbs. Each scFv showed the same specificity of binding as the original MAb, and both scFv bound with a 10- to 20-fold lower efficiency compared with the IgG. This was most likely a reflection of the increased avidity of the divalent IgG compared with the generally monovalent scFv. Interestingly, when tested in neutralization assays, the scFvs showed an efficiency of neutralization similar to that of the IgG (Fig. 4A). This was similar to the effect seen for neutralization by purified Fab and IgG of the MAb 8 (Wikoff et al., 1994). The reduced efficiency of neutralization by the scFv-8 mutants indicated that the affinity of virus binding affected its ability to neutralize.

**CDR contribution to binding**

To examine the contributions of the various sequences in the scFv-8 paratope to antibody binding, we used the cryoelectron microscopic structure of MAb 8 Fab bound to the CPV capsid as a model (Wikoff et al., 1994). The H1 and H3 CDRs appeared to make extensive contacts with the epitope, while H2, L1, and L3 made only minor contacts (Fig. 9). We confirmed this model, as mutations within either the H1 or H3 CDR reduced the affinity of the scFv binding, while mutants of CDR H2 had no effect (Fig. 5).

MAb 8 covers portions of loops 1, 2, and 3 from three symmetry-related VP2 molecules, and most closely contacted residues 87, 300, and 301 (Fig. 9) (Strassheim et al., 1994; Parker and Parrish, 1997). The 300 Ala to Asp mutant capsid structure shows that the Asp side chain forms a new salt bridge with Arg 81 of an adjacent VP2, changing the conformation of the surface loop (Fig. 9B) (Llamas-Saiz et al., 1996). The side chain of the Asn 302 projects up from the capsid surface and likely contacts...
both heavy-chain CDR1 and CDR3, explaining why changing that residue to Asp prevented antibody binding. These results show that the conformation of the epitope is important for MAb 8 binding and that single amino acids within the epitope do not affect the interaction with antibody unless they change critical contacts.

HA inhibition

The scFv-8 inhibited HA with only 10-fold reduced efficiency compared with MAb 8. However, scFv-14 showed no HA activity even at 400 nM, whereas 0.125 nM MAb 14 inhibited HA (Table 1). This difference between scFv-14 and MAb 14 appeared to be related to the size or valency of the binding complex, because adding an anti-Fab antibody gave HA inhibition at about 10 nM scFv-14. The difference between scFv-8 and scFv-14 may reflect the distance (~40 Å) between site A bound by scFv-14 and the sialic acid binding site, while scFv-8 binds at a distance of less than 10 Å and thus would still block sialic acid binding (Tresnan et al., 1995).

Neutralization mechanisms

Over 30 MAbs that we have derived which recognize intact CPV capsids neutralize the virus with varying efficiency (Parrish and Carmichael, 1983; Strassheim et al., 1994), as did the scFvs prepared here. Some of the neutralization could be explained by aggregation of the capsids by the scFv or MAb (Fig. 6). The mechanisms of aggregation by the scFv were not defined, but are most

<table>
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<th>Antibody or scFv</th>
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</table>

a scFv-8 mutants H1, H3, and H1 and H3 contained changes in the V<sub>H</sub> CDR 1 and/or CDR 3.

b The minimum concentration of scFvs or MAbs required to inhibit viral HA.

**TABLE 1**

Inhibition of Viral Hemagglutination by scFvs and MAbs

**FIG. 4.** (A) Neutralization by scFv compared with that by intact IgG of the same MAb. (B) Neutralization by three mutants in the heavy-chain CDR1 and CDR3 of scFv-8 compared with that by wild-type scFv-8. Titrations were performed in NLFO cells, and the TCID<sub>50</sub> remaining after incubation in the presence of dilutions of the antibody are shown.

**FIG. 5.** (A) Mutations prepared within the scFv-8 heavy-chain CDRs. Amino acid residues mutated to Ala (A) are shown in bold. (B) Effects of scFv-8 CDR mutants on the binding to CPV capsids. Biotinylated scFv-8 was incubated with CPV in the presence of varying amounts of unlabeled scFv-8 or mutant derivatives. Biotinylated scFv-8 bound was detected with HRP-ExtrAvidin and ABTS.
likely the result of the presence of a proportion of bivalent scFv in the preparations resulting from intermolecular associations of the scFv (Raag and Whitlow, 1995), or perhaps to some other effect of the scFv binding which caused the virus to aggregate. Both scFvs and MAbS increased binding of CPV capsids to NLFK and A72 cells, perhaps as a result of increased association of the virus aggregates. It is therefore likely that neutralization occurs through aggregation as well as through interference with capsid transport or uncoating within cells.

Previous studies have defined neutralizing epitopes within the capsids of CPV, pig parvovirus, and the human parvovirus B19, and those were found within the capsid surface, similar to the A and B sites described here, as well as in the VP2 N-terminus of CPV and in the VP1-unique region of B19 (Brown et al., 1992; Langeveld et al., 1993; Saikawa et al., 1993; Casal et al., 1995; Kamstrup et al., 1998). This is the first study to investigate the details of the antibody-binding and neutralization mechanisms of parvoviruses. The mechanisms are generally similar to those reported for the neutralization of other nonenveloped and enveloped viruses, indicating that there are only a limited number of processes of neutralization for most viruses (Outlaw and Dimmock, 1991; Smith, 1993; Verdaguer et al., 1997; Che et al., 1998; Fleury et al., 1999; Sattentau et al., 1999; Smith and Baker, 1999).

MATERIALS AND METHODS

Cells and viruses

NLFK cells and canine A72 cells were grown in a 1:1 mixture of McCoy’s 5A and Leibovitz L15 media with 5% fetal bovine serum (FBS). CPV-d and FPV-b were derived from the infectious plasmid clones by transfection of NLFK cells (Parrish, 1991). Capsids were purified using previously described procedures involving polyethylene glycol precipitation, followed by repeated banding on 10–40% sucrose gradients (Agbandje et al., 1993; Weichert et al., 1998).

Construction of the single-chain antibodies (scFv)

Total RNA was extracted from hybridoma cell lines secreting MAb 8 or MAb 14 (Parrish and Carmichael, 1983; Strassheim et al., 1994) by the RNAAgents Total RNA Isolation System (Promega, Madison, WI). First-strand cDNA was synthesized using an oligo(dT) primer and MMLV reverse transcriptase, and then the Vh and Vl regions were amplified by polymerase chain reaction (PCR), with primers either predicted by the consensus murine κ-chain sequence or from the Ig-Prime Kit (Novagen, Madison, WI) (Table 2). PCR products were cloned into pT7Blue T-vector (Novagen) and sequenced using automated DNA sequencing. The CDRs of each of the Vh and Vl regions were assigned by comparison with Kabat database (Fig. 1) (Kabat et al., 1991).

To construct scFvs the cloned Vl and Vh regions were reamplified by PCR with the use of primers designed

![FIG. 6. Aggregation of 125I-labeled CPV capsids by scFv or MAb after incubation at various ratios for 1 h at 20°C. The mixtures were separated in 10–40% (w/v) sucrose gradients. The 125I-CPV in the pellet and in fractions below the free virus peak were considered to be viral aggregates. Free capsids or capsids incubated with rabbit polyclonal antiserum (diluted 1:400) were included as parallel controls.](image)

![FIG. 7. Binding of 125I-labeled CPV capsids to (A) NLFK cells at 4°C or (B) to A72 cells at 37°C. After incubation with scFv or MAb at the ratios shown for 1 h at 20°C, the capsids were added to the cells and incubated for 1 h. The cells were washed and the bound 125I-labeled CPV counted.](image)
from the sequences obtained from each variable region. A sequence encoding (Gly4Ser)3 (Bird et al., 1988; Huston et al., 1988) was used to link the V<sub>L</sub> and V<sub>H</sub> regions by overlapping extension PCR (Horton et al., 1989), and then the products were cloned in the plasmid pGEM-T (Promega). The complete scFv sequences isolated with MscI and XhoI restriction enzymes were ligated into the vector pET22b(+)(Novagen) (Fig. 2), adding the N-terminal pelB leader sequence (Lei et al., 1987) and the six C-terminal histidines to each scFv. The scFvs derived from MAb 8 and MAb 14 were named scFv-8 and scFv-14, respectively.

Site-directed mutagenesis of CDR sequences of scFv-8

The contact residues in the heavy-chain CDR1 (H1), CDR2 (H2), or CDR3 (H3) of scFv-8 were altered by site-directed mutagenesis using specific oligonucleotide primers and uracil-containing M13 DNA, as described by Kunkel et al. (1987) (Fig. 5A). Double-mutant scFvs were prepared by recombinations between the different CDRs using intervening restriction enzyme sites (Fig. 5A). The mutated sequences were reintroduced into pET22b(+) for expression.

Expression and purification of scFvs

The E. coli strain BL21(DE3) (Novagen) containing the scFv plasmids was grown at 30°C to an OD<sub>600</sub> of 0.8, 0.5 mM IPTG was added, and cells were cultured for 12 h at 25°C. After washing with 0.1 volume of 30 mM Tris–HCl (pH 8.0), 20% sucrose, and 1 mM EDTA, the bacteria were extracted with ice-cold 5 mM MgSO<sub>4</sub> to prepare the periplasmic fraction (Ausubel et al., 1989). That was made up to 20 mM Tris-HCl (pH 7.9), 500 mM NaCl, and 5 mM imidazole, and loaded onto a Ni<sup>2+</sup> His-binding column (Novagen). After washing with 60 mM imidazole, the scFvs were eluted with 1 M imidazole and dialyzed.
against phosphate-buffered saline (PBS, pH 7.4). The purity of the scFv preparation was evaluated by electrophoresis on 12% SDS–PAGE and concentrations were determined by Micro BCA Protein Assay Reagent Kit (Pierce Chemicals, Rockford, IL).

**ELISA testing of scFv binding**

Wells of 96-well plates were incubated at 4°C with 5 μg/ml of CPV or FPV empty capsids or of capsids boiled for 5 min. After incubation with 1% (w/v) BSA in PBS, the plates were washed and incubated with varying dilutions of the scFv for 1 h, and then bound scFv was detected with goat anti-mouse IgG (Fab specific) conjugated with horseradish peroxidase (HRPO) and the substrate 2,2'-azino-bis(3-ethylbenzthiazoline 6-sulfonic acid) diammonium (ABTS). MAb 8 and MAb 14 were purified from hybridoma supernatants by HiTrap Protein G column (Amersham Pharmacia Biotech, Piscataway, NJ).

Relative binding affinities of scFv-8 mutants were tested with the use of competition ELISA. The wild-type scFv-8, biotinylated with the use of Sulfo-NHS-LS-biotin (Pierce Chemicals), was mixed with varying concentrations of unlabeled wild-type or mutant scFv-8, added to 96-well plate wells coated with CPV empty capsids, and incubated for 1 h. After washing, the biotinylated scFv-8 bound was detected by the use of HRPO-ExtrAvidin (Sigma, St. Louis, MO) and ABTS.

**Viral neutralization assays**

Purified scFvs or MAbs were diluted and incubated with CPV-d for 1 h at 20°C. Each mixture was then titrated in a TCID₅₀ assay by the use of A72 cells or NLFK cells in 96-well plates. After 48 h incubation at 37°C, the cells were fixed with 2.5% paraformaldehyde, and infected cells were detected with the use of rabbit polyclonal antiserum against CPV capsids, HRPO-conjugated goat anti-rabbit IgG, and the substrate aminoethyl carbazole (AEC). TCID₅₀ were calculated by the method of Reed and Muench (1938).

**Inhibition of HA**

Purified scFvs or MAbs were diluted and incubated with 4 HA units of CPV at 20°C for 1 h. Equine erythrocytes (0.5% v/v) in 20 mM Bis–Tris (pH 6.2) and 150 mM NaCl at 4°C were added and the plates incubated at 4°C (Barbis et al., 1992; Tresnan et al., 1995). To test the effect of crosslinking the scFvs, they were incubated with an equal molarity of goat anti-mouse IgG (Fab specific) for 1 h at 20°C, diluted, and incubated with CPV-d.

**Cell binding and entry**

Binding of capsids to NLFK cells or A72 cells was assayed with CPV-d labeled with ¹²⁵I (Barbis et al., 1992). Labeled capsids (0.1 μg, 1.3 x 10¹⁰ particles) were incubated with purified scFvs or MAbs at 1:5, 1:1, or 1:0.2 ratios of capsid VP2 to antibody-combining site at 20°C for 1 h. The cells were disassociated from culture flasks with a nonenzymatic disassociation solution and washed with DMEM. The CPV/antibody mixture was incubated in DMEM and 0.1% bovine serum albumin with 1.3 x 10⁵ NLFK cells at 4°C, or with A72 cells at 37°C. After 1 h the cells were washed in the same buffer and the bound ¹²⁵I was counted.

CPV capsids (40 μg/ml) were mixed and incubated with the scFv or MAb at 20°C for 1 h, then added to NLFK or A72 cells. After 30-min incubation at 37°C the cells were fixed with 2.5% paraformaldehyde in PBS and stained with an FITC-conjugated rabbit polyclonal IgG against the CPV capsid. The cells were observed by confocal microscopy and optical sections collected from the center of the cells.

**Aggregation of capsids by scFvs or MAbs**

scFvs or MAbs were incubated at 20°C for 1 h with ¹²⁵I-labeled CPV-d at 1:1, 1:0.2, or 1:0.04 ratios of capsid VP2 to scFv or MAb combining site. Mixtures were loaded onto 10 to 40% sucrose gradients in DMEM and centrifuged in a SW41 rotor at 36,000 rpm for 2.5 h, the

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**TABLE 2**

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</tbody>
</table>

* Primers MuIgV₅'-E, MuIgV₅'-C, and MuIgG₃'-2 were those isolated in the cDNA clones prepared.
gradients were fractionated, and the $^{125}$I DPM in each fraction or in the pellet was determined. The aggregated capsids were determined as those in the pellet or in the fractions below the peak of free virus.

ACKNOWLEDGMENTS

Wendy Weichert and Gail Sullivan provided expert technical assistance. This work was supported by National Institutes of Health Grant AI33468.

REFERENCES


