

Amendment history:

- [Correction](#) (December 1992)

Unique region of the minor capsid protein of human parvovirus B19 is exposed on the virion surface.

S J Rosenfeld, ... , G Bansal, M S Collett

J Clin Invest. 1992;**89**(6):2023-2029. <https://doi.org/10.1172/JCI115812>.

Research Article

Capsids of the B19 parvovirus are composed of major (VP2; 58 kD) and minor (VP1; 83 kD) structural proteins. These proteins are identical except for a unique 226 amino acid region at the amino terminus of VP1. Previous immunization studies with recombinant empty capsids have demonstrated that the presence of VP1 was required to elicit virus-neutralizing antibody activity. However, to date, neutralizing epitopes have been identified only on VP2. Crystallographic studies of a related parvovirus (canine parvovirus) suggested the unique amino-terminal portion of VP1 assumed an internal position within the viral capsid. To determine the position of VP1 in both empty capsids and virions, we expressed a fusion protein containing the unique region of VP1. Antisera raised to this protein recognized recombinant empty capsids containing VP1 and VP2, but not those containing VP2 alone, in an enzyme-linked immunosorbent assay. The antisera immunoprecipitated both recombinant empty capsids and human plasma-derived virions, and agglutinated the latter as shown by immune electron microscopy. The sera contained potent neutralizing activity for virus infectivity in vitro. These data indicate that a portion of the amino terminus of VP1 is located on the virion surface, and [...]

Find the latest version:

<https://jci.me/115812/pdf>



Unique Region of the Minor Capsid Protein of Human Parvovirus B19 Is Exposed on the Virion Surface

Stephen J. Rosenfeld,* Kohji Yoshimoto,* Sachiko Kajigaya,* Stacie Anderson,* Neal S. Young,* Anne Field,† Paul Warrener,‡ Geetha Bansal,‡ and Marc S. Collett§

*Cell Biology Section, Clinical Hematology Branch, National Heart, Lung and Blood Institute, Bethesda, Maryland 20817;

†Public Health Laboratory Service Virus Reference Laboratory, London NW9 5HT, United Kingdom;

and §Medimmune, Inc., Gaithersburg, Maryland 20878

Abstract

Capsids of the B19 parvovirus are composed of major (VP2; 58 kD) and minor (VP1; 83 kD) structural proteins. These proteins are identical except for a unique 226 amino acid region at the amino terminus of VP1. Previous immunization studies with recombinant empty capsids have demonstrated that the presence of VP1 was required to elicit virus-neutralizing antibody activity. However, to date, neutralizing epitopes have been identified only on VP2. Crystallographic studies of a related parvovirus (canine parvovirus) suggested the unique amino-terminal portion of VP1 assumed an internal position within the viral capsid. To determine the position of VP1 in both empty capsids and virions, we expressed a fusion protein containing the unique region of VP1. Antisera raised to this protein recognized recombinant empty capsids containing VP1 and VP2, but not those containing VP2 alone, in an enzyme-linked immunosorbent assay. The antisera immunoprecipitated both recombinant empty capsids and human plasma-derived virions, and agglutinated the latter as shown by immune electron microscopy. The sera contained potent neutralizing activity for virus infectivity *in vitro*. These data indicate that a portion of the amino terminus of VP1 is located on the virion surface, and that this region contains intrinsic neutralizing determinants. The external location of the VP1-specific tail may provide a site for engineered heterologous epitope presentation in novel recombinant vaccines. (*J. Clin. Invest.* 1992; 89:2023–2029.) Key words: neutralization • antibodies • vaccine • epitope • immunoprecipitation

Introduction

Parvoviruses are small, unenveloped, single-stranded DNA viruses widely distributed in the animal kingdom. The human B19 parvovirus causes several diseases in humans (1). In the normal host, the virus is rapidly cleared by a brisk antibody response, and illness is usually mild and self limited. In other

hosts characterized by a susceptible target cell population or an inadequate immune system, B19 parvovirus causes significant morbidity and mortality. The target cell for B19 infection is an immature erythrocyte progenitor at about the CFU-E stage of development. The virus is directly toxic to this cell and completely ablates erythroid colony formation in bone marrow cultures (2). In patients with increased requirements for erythrocytes or shortened red cell survival, acute B19 infection causes the rapid development of severe anemia and the syndrome of transient aplastic crisis (TAC)¹ (1). TAC can be treated supportively with transfusions and resolves with the development of a specific antibody response. In immunocompromised hosts, the antibody response to parvovirus is inadequate, and persistent infection causes the gradual onset of a severe anemia. Persistently infected patients can be treated effectively with immunoglobulins, but in the face of unremitting or severe immunodeficiency, such as occurs in infection with the human immunodeficiency virus, they often relapse (3). The developing fetus is also susceptible to B19 parvovirus, and severe anemia leads to spontaneous abortion or hydrops fetalis. Hydrops can be treated with intrauterine exchange transfusions, but it may not be recognized in time to avoid fetal death (4). In all three of these groups, therapy is expensive and carries significant risks, making a preventive strategy attractive. Normal clearance of the virus appears to be achieved almost entirely through the humoral arm of the immune response, and effective vaccines have been developed for the related canine and feline parvoviruses (5), suggesting that prevention of B19 parvovirus infection can be achieved through use of a vaccine.

The structural proteins of B19 are encoded by two overlapping reading frames on the right side of the viral genome. The major capsid protein (VP2) is translated from an RNA generated by a splicing event that removes the 5' 678 nucleotides of the minor capsid protein (VP1) coding region. Thus, the amino acid sequences of the two proteins are identical except for 226 residues on the amino terminus of VP1. 60 copies of the capsid proteins assemble into an icosahedral shell with ~ 95% VP2 and 5% VP1. The structural distribution of VP1 and the position of its unique tail are unknown. In many parvoviruses, this tail has a disproportionate number of basic amino acids and has been assumed to be located within the capsid, where it would partially neutralize the negative charge of the viral DNA (6, 7).

Address correspondence and reprint requests to Dr. Stephen Rosenfeld, Building 10 Room 7C108, National Heart, Lung and Blood Institute, National Institutes of Health, 9000 Rockville Pike, Bethesda, Maryland 20817.

Received for publication 11 October 1991 and in revised form 31 January 1992.

The Journal of Clinical Investigation, Inc.
Volume 89, June 1992, 2023–2029

1. Abbreviations used in this paper: STE, 150 mM NaCl, 10 mM Tris-HCl, pH 7.4, 1 mM EDTA; TAC, transient aplastic crisis; VP1, minor capsid protein; VP2, major capsid protein.

We have previously reported the production of empty viral capsids in a baculovirus-based expression system (8). Recombinant structural proteins self assembled into empty capsids indistinguishable from virions by electron microscopy. In addition, empty capsids could be used to detect virus-specific antibody in ELISAs, and animals immunized with empty capsids produced a strong antibody response that cross-reacted with virus, suggesting that virions and empty capsids are antigenically similar. We discovered that while VP1 was not necessary for capsid self assembly, capsids made from VP2 alone failed to elicit a neutralizing antibody response when inoculated into rabbits or mice. Empty capsids containing normal amounts of VP1 elicited neutralizing titers in rabbits that were similar to those seen in convalescent phase human sera. Despite this absolute requirement for VP1 to generate a neutralizing response when immunizing with empty capsids, several neutralizing epitopes have been mapped to regions within VP2 (9–11). Thus, VP1 appears to modify the antigenicity of epitopes on VP2. We report here the identification of additional neutralizing regions on this unique amino-terminal tail of VP1 and present evidence that this tail lies on the outer surface of both empty capsids and virions.

Methods

Molecular cloning and gene expression of the unique amino-terminal region of VP1. To express the VP1 protein-specific region of B19, DNA from the serum of a viremic patient was extracted with phenol in the presence of sodium dodecyl sulfate. A 681-bp DNA fragment encompassing nucleotides 2444 to 3124 of the B19 genome (12) was amplified by the polymerase chain reaction using primers 5'-GGAAGATCT-CATGAGTAAAGAAAAGTGGCAAATGG-3' and 5'-CGCGGGATC-CAAGCTTGGGTATTTTTCTGAG-3'. Using the engineered BglII and BamHI restriction sites in the primers, amplified DNA was cloned into the transcription vector pSP72 (Promega Biotech, Madison, WI) and then completely sequenced. Homology with the previously reported sequence of this region of the parvovirus genome (12) was 99.3% (676 of the 681 nucleotides were identical; two amino acids were changed). This VP1-specific sequence was subcloned into an *Escherichia coli* plasmid expression system (13). The VP1-specific sequence was inserted (either as a single copy or as a tandem repeat) downstream of the tac promoter and sandwiched between sequences coding for the amino terminal 23 amino acids of the bacteriophage lambda cro protein and a segment encoding the last 17 residues of beta-galactosidase. The resultant plasmids, designated pCro VP1-227M (single copy) and pCro VP1-227D (tandem repeat) were used to transform *E. coli* strain NF1829 by standard procedures (14). Induction of transformed cultures with isopropyl-beta-D-thiogalactoside (IPTG) resulted in production of the respective 33-kD and 64-kD fusion proteins. These fusion proteins, which are produced in bacteria as insoluble aggregates, were partially purified by previously described procedures (15). Induced bacteria were harvested, treated with lysozyme (400 µg/ml, 4°C for 15 min), adjusted to 1% Triton X-100, and then sonicated. MgCl₂ and DNase I were added to the resulting lysate to a final concentration of 5 mM and 10 µg/ml, respectively. After incubation at 37°C for 30 min, the mixture was layered onto a 35% sucrose solution in an SW28 ultracentrifuge tube and centrifuged at 50,000 g for 60 min. Pelleted material was resuspended in STE (150 mM NaCl, 10 mM Tris-HCl, pH 7.4, 1 mM EDTA) and repelleted by centrifugation at 10,000 g for 10 min. The pellet was resuspended in STE to a protein concentration of 2–5 mg/ml and then adjusted to 1% SDS, 2% 2-mercaptoethanol. After boiling for 5 min, this solution was extensively dialyzed against STE (pH 8.5), 0.01% 2-mercaptoethanol, and 10% sucrose.

Rabbit antisera. Dialyzed fusion proteins (1 mg) were emulsified in complete Freund's adjuvant and administered intramuscularly to rab-

bits. The animals were boosted with 0.5 mg of protein emulsified in Freund's incomplete adjuvant 3 wk after the initial immunization, and two additional booster immunizations were given at 5 and 7 wk. Animals were bled 2 wk after booster immunizations and samples were evaluated for antibodies by radioimmunoprecipitation.

Immunoblotting. Human serum containing B19 virions was boiled in Laemmli sample buffer, electrophoresed through an 8% SDS-polyacrylamide gel, and electrophoretically transferred to PVDF membranes (United States Biochemical Corp., Cleveland, OH) at 100 mA for 16 h. Transfer buffer contained 25 mM Tris and 192 mM glycine. Membranes were blocked with 1% blocking solution (United States Biochemical Corp.), incubated with antisera diluted 1:500, and incubated with alkaline phosphatase conjugated goat anti-rabbit immunoglobulin (Gibco BRL Life Technologies, Gaithersburg, MD). Antibody incubations were performed for 1 h at room temperature, and the membranes were washed three times with a salt buffer containing 0.1% Tween 20 between incubations. After soaking the membranes with substrate buffer (100 mM Tris, pH 9.5, 100 mM NaCl, 50 mM MgCl₂), bound antibody was detected with a substrate solution containing nitroblue tetrazolium (NBT) (Gibco BRL Life Technologies) and BCIP (Gibco BRL Life Technologies).

Antigen-down ELISA. *Spodoptera frugiperda* Sf9 cells (American Type Culture Collection, Rockville, MD) were infected with recombinant baculoviruses bacVP1 and bacVP2, combined in ratios of 0:1, 1:1, and 10:1. Cells were cultured in Grace's insect medium (Gibco BRL Life Technologies) in room air, 95% humidity, at 27°C. Cells were harvested 3 d after inoculation and lysed by Dounce homogenization in a solution containing 5 mM 2-mercaptoethanol and 0.2% Triton X-100. Lysates were centrifuged at 100,000 g for 16 h over 40% sucrose in HBSS. Pellets were resuspended in CsCl at an initial density of 1.31 g/ml and centrifuged at 100,000 g in an SW41 rotor for 40 h at 18°C. Visible bands containing empty capsids were collected and dialyzed into HBSS, and the presence of B19 structural proteins was verified by SDS-PAGE and immunoblot.

ELISA plates were prepared by attaching 1 µg of purified empty capsids to each well of Immulon-2 96-well microtiter plates (Dynatech Laboratories Inc., Chantilly, VA) by overnight incubation in PBS at 4°C. 100 µl of diluted rabbit antiserum was added to each well after blocking with PBS containing 3% bovine serum albumin. Plates were washed with PBS containing 0.05% Tween 20 and incubated with peroxidase-labeled goat anti-rabbit antibodies (Gibco BRL Life Technologies) for 1 h at room temperature. After a second wash, 200 µl of 0.1 M citrate buffer containing *o*-phenylenediamine and H₂O₂ was added to each well. The enzyme reaction was stopped with 4 N H₂SO₄ and the light absorbance at 490 nm was measured with an automated microplate reader (Bio-Rad Laboratories, Richmond, CA).

Immunoprecipitation. For empty capsids, Sf9 cells were coinfecting with recombinant baculoviruses bacVP1 and bacVP2 and cultured for 2 d as described above. After preincubation in methionine-free medium for 2 h, cells were cultured for 5 h in the presence of 100 µCi/ml [³⁵S]methionine. Labeled empty capsids were isolated and purified as described above and resuspended in HBSS.

Bead solution was prepared by combining protein A Sepharose (CL-4B; Pharmacia LKB Biotechnology, Piscataway, NJ) 10% (vol/vol) in PBS with 1 mg/ml BSA. Washing solution was PBS with 1 mg/ml BSA. HBSS containing labeled empty capsids was precleared with preimmune rabbit serum, and immunoprecipitation was performed by the addition of 10 µl of immune rabbit serum. All serum incubations and antibody precipitations were performed for 1 h at 4°C with constant agitation. After the last precipitation, beads were washed three times with washing solution, resuspended in Laemmli sample loading buffer and boiled for 10 min. The supernatant was electrophoresed in 8% SDS-polyacrylamide to separate the capsid proteins.

For full virions, serum containing infectious virus was diluted 1:10 with PBS, and 10 µl was precleared by incubation with beads alone, followed by preimmune rabbit serum. Immunoprecipitation was performed as described above. After the last wash, beads were resuspended in 1/3 N NaOH and boiled for 5 min. Samples were neutralized with an

equal volume of 1/3 N HCl and blotted onto Nytran filters (Schleicher and Schuell Inc., Keene, NH) using a manifold (Slot Blot; Schleicher and Schuell). Filters were UV-cross-linked in an oven (StrataLink; Stratagene Inc., La Jolla, CA), prehybridized at 42°C for at least 2 h, and hybridized at 42°C for 12 h in 50% formamide, 5× standard saline citrate (SSC), 1× Denhardt, 0.5% sodium dodecyl sulfate, 10% Dextran, and 100 µg/ml salmon sperm DNA. Virus-specific DNA probes were made by excising the viral sequence from pYT103c, a plasmid containing a nearly full-length B19 clone, and labeling with a random hexanucleotide labeling kit (Stratagene Inc.). After hybridization, filters were washed sequentially with 2× SSC, 2× SSC/1% SDS, 0.1× SSC/1% SDS, and 0.1× SSC. Prehybridization, hybridization, and washing were performed in a Hybridiser oven (Techne Inc., Princeton, NJ).

DNase treatment. 1 µl of infectious serum was diluted to 10 µl with PBS containing 6 mM MgCl₂ and 0.1 mM CaCl₂. 1 µl of RQ-1 DNase (Promega Biotech) was added and the mixture was incubated for 30 min at 37°C. Loss of DNase activity caused by the addition of serum components was assessed by digesting template DNA that had been added to similarly diluted normal human serum.

Electron microscopy. Empty capsids containing VP1 and VP2 were prepared as described above. After ultracentrifugation through 40% sucrose, the pellet was resuspended in HBSS and diluted 1:25 in distilled water. Capsids were negatively stained using 3% phosphotungstic acid, pH 6.5. Grids were examined to determine if capsids were intact.

Human serum containing infectious virions was diluted and stained as described above. Immune electron microscopy was performed to determine the antibody activity of immune and preimmune rabbit antisera using previously described techniques (8).

Neutralization assays. Antibody-containing serum was heated to 56°C for 30 min to inactivate complement. Serum was then mixed with a 1:20 dilution of human serum containing infectious B19 parvovirus and incubated for 2 h at 4°C. Bone marrow was harvested from a normal volunteer under a protocol approved by the National Heart, Lung, and Blood Institute Institutional Review Board, and mononuclear cells were isolated by centrifugation over a Ficoll-Hypaque gradient. Bone marrow mononuclear cells were incubated with the virus/

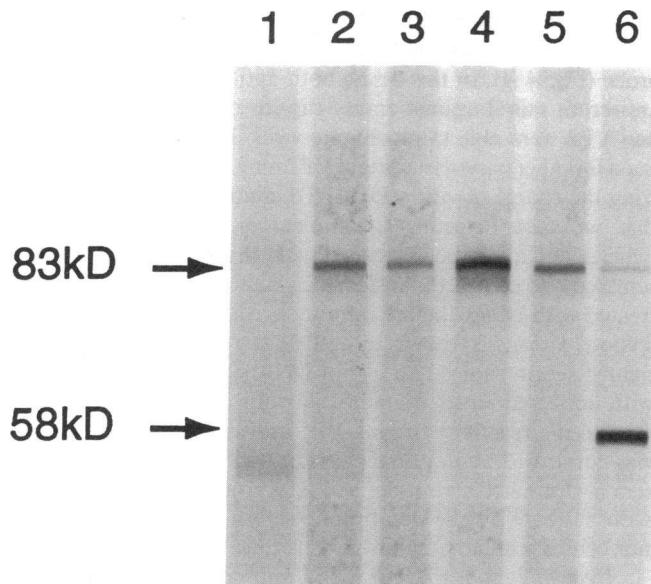


Figure 1. Specificity of rabbit antisera for serum-derived VP1 protein. All four sera (lanes 2–5) detected a protein of the expected 83-kD size in serum containing virus. The more abundant 58-kD VP2 species was detected by antiserum raised against empty capsids containing both VP1 and VP2 (lane 6), and preimmune serum failed to detect either protein (lane 1).

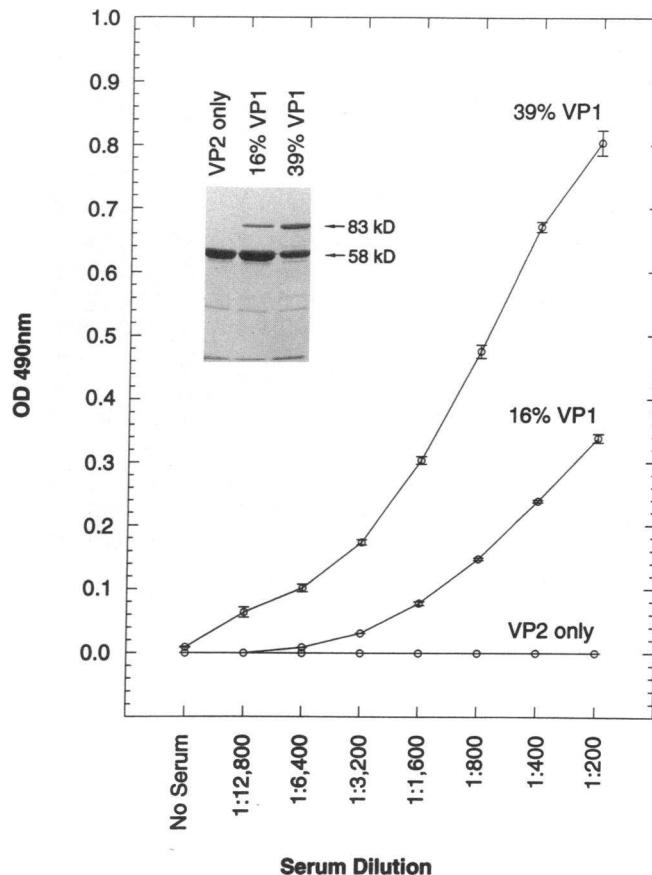


Figure 2. Reactivity of anti-VP1 serum against empty capsids containing varying amounts of VP1. Empty capsids containing varying proportions of VP1 were produced by coinfecting SF9 cells with bacVP1 and bacVP2 in different multiplicities of infection. (*Inset*) Ratio of VP1 to VP2 in each capsid preparation was assessed by Coomassie staining of purified empty capsids separated into component proteins by SDS-PAGE. Proteins were quantified by densitometry. (*Graph*) An ELISA was used to assess reactivity of antiserum R3 (raised in rabbit 3) against empty capsids with varying ratios of VP1 to VP2.

serum preparation at a final concentration of 3×10^6 cells/ml for 1 h at 4°C and plated after dilution in standard tissue culture medium for late erythroid progenitors (CFU-E) containing 0.8% methylcellulose, 30% fetal calf serum, 1% BSA, 10^{-3} M beta-mercaptoethanol, and 1 U/ml recombinant erythropoietin (Amgen Biologicals, Thousand Oaks, CA). Cultures were incubated for 7–8 d at 37°C, 95% humidity, and colonies were counted visually. All cultures were performed in duplicate, and controls included virus without serum, virus with known neutralizing serum, and cells without virus or serum.

Results

Antisera directed against the sequences unique to the VP1 capsid protein. The DNA sequence representing the amino terminal 226 amino acids of VP1 was cloned into an *E. coli* expression system and the resultant recombinant fusion proteins were used to immunize rabbits. Two rabbits each were immunized with either the croVP1-227M monomeric or the croVP1-227D dimeric polypeptide. VP1 specificity of harvested sera was confirmed by immunoblot against serum-derived viral proteins. All four antisera detected a protein of the expected

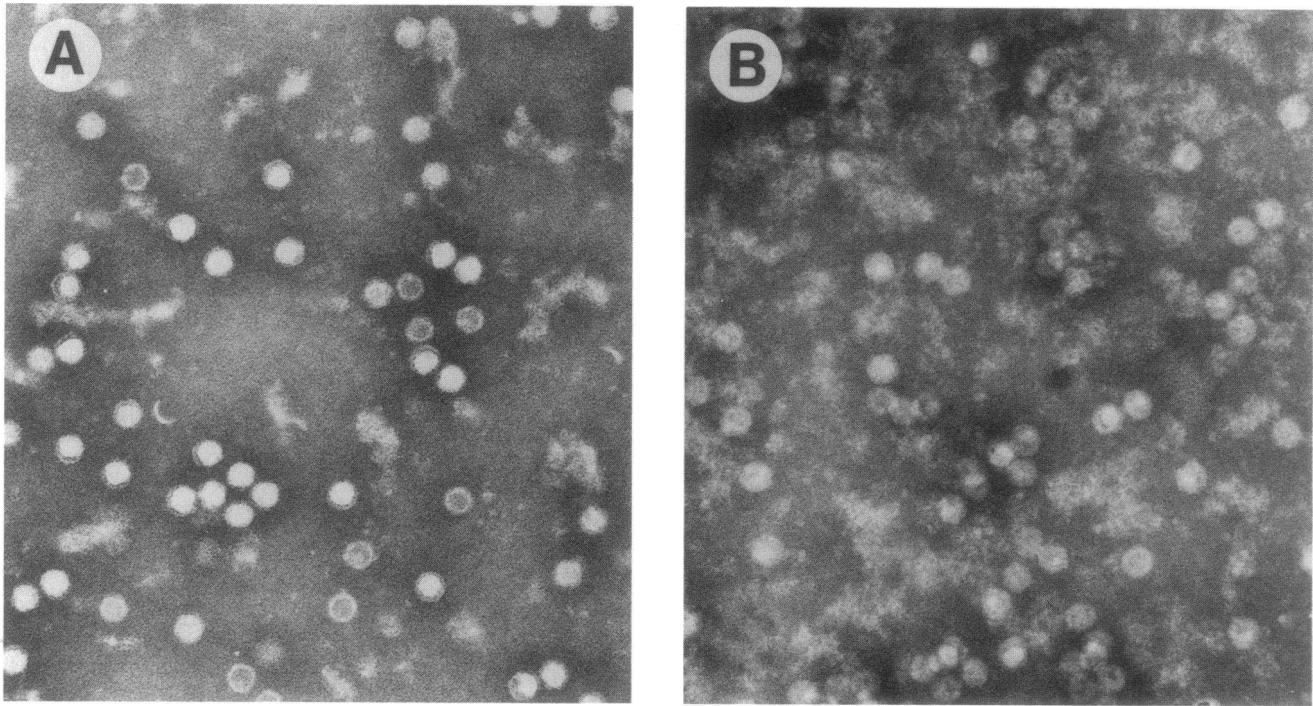


Figure 3. Electron microscopy of sedimented empty capsids. Direct electron microscopy of empty capsids containing VP1 and VP2 (A) or VP2 alone (B), expressed in a baculovirus expression system and centrifuged through 40% sucrose.

size of 83 kD and failed to detect the smaller but more abundant 58-kD VP2 protein (Fig. 1).

An enzyme-linked immunosorbent assay was used to confirm the specificity of the anti-VP1 sera against nondenatured capsids. Antisera failed to react with empty capsids containing only VP2, but reacted strongly with empty capsids containing two different concentrations of VP1 (Fig. 2). Reactivity was proportional to the VP1 content of the capsids.

Immunoprecipitation of empty capsids. Initial screening of the antisera by radioimmunoprecipitation suggested that the unique region of VP1 was on the external surface of recombinant empty capsids. We compared immunoprecipitation using the VP1 specific sera with immunoprecipitation using antisera raised against intact empty capsids containing both VP1 and VP2. Labeled capsids were produced in Sf9 cells infected with recombinant baculoviruses encoding the VP1 and VP2 sequences. To avoid detection of free proteins or incompletely assembled capsids, capsids were purified from cell lysates by centrifugation through a sucrose cushion. This technique routinely produces empty capsids with a uniform sedimentation coefficient of 70S upon rate-zonal centrifugation. Integrity of sedimented capsids was confirmed by electron microscopy, which showed that over 99% of capsids were intact (Fig. 3). Both antisera raised to the VP1-specific region and antisera raised against empty capsids containing both structural proteins were able to precipitate capsids (Fig. 4 B).

Immunoprecipitation of virions. To determine if the position of the unique VP1 region was similar in recombinant capsids and DNA-containing virions, we performed a second set of immunoprecipitation experiments. Because B19 parvovirus can only be reliably propagated in fresh human bone marrow culture (16), conventional immunoprecipitation techniques that follow metabolic labeling of the virion proteins are cum-

bersome, and high specific activity is not achieved. In addition, like other parvoviruses, B19 produces both empty capsids and intact virions during the course of infection. These species appear to have similar but not identical antigenic properties, and they would be impossible to distinguish by detection of metabolically labeled proteins. To circumvent these problems, human serum known to contain a high level of infectious virus was immunoprecipitated and captured virus was measured by DNA slot blotting and hybridization with a B19-specific DNA probe (Fig. 4 A). In this assay, both anti-VP1 antiserum and antiserum raised against empty capsids containing both VP1 and VP2 were able to precipitate virus. It has been hypothesized for other parvoviruses that VP1 may play a role in neutralizing the charge of virion DNA. To control for the possibility that we were detecting incomplete or fragmented virions (where VP1 might be associated with virion DNA but not assembled into complete capsids) the virus-containing serum was treated with DNase before immunoprecipitation (DNA in infectious virions is known to be DNase resistant [2]). DNase-treated serum showed no loss of DNA signal when compared with untreated serum (data not shown).

Specific reactivity of anti-VP1 antisera and intact virions was confirmed by immune electron microscopy. Rabbit antisera agglutinated serum-derived B19 virions and coated them with antibody, while preimmune serum neither agglutinated nor coated similarly prepared virus (Fig. 5).

Neutralization. All four rabbit antisera produced after immunization with the VP1-specific fusion peptide or its dimer showed virus neutralizing activity in vitro. To assess the relative neutralization titers of these antisera, increasing dilutions were assayed for neutralization ability (Fig. 6). All sera maintained their ability to neutralize virus to at least a 1/10 dilution, and three of the four antisera had measurable neutralizing activ-

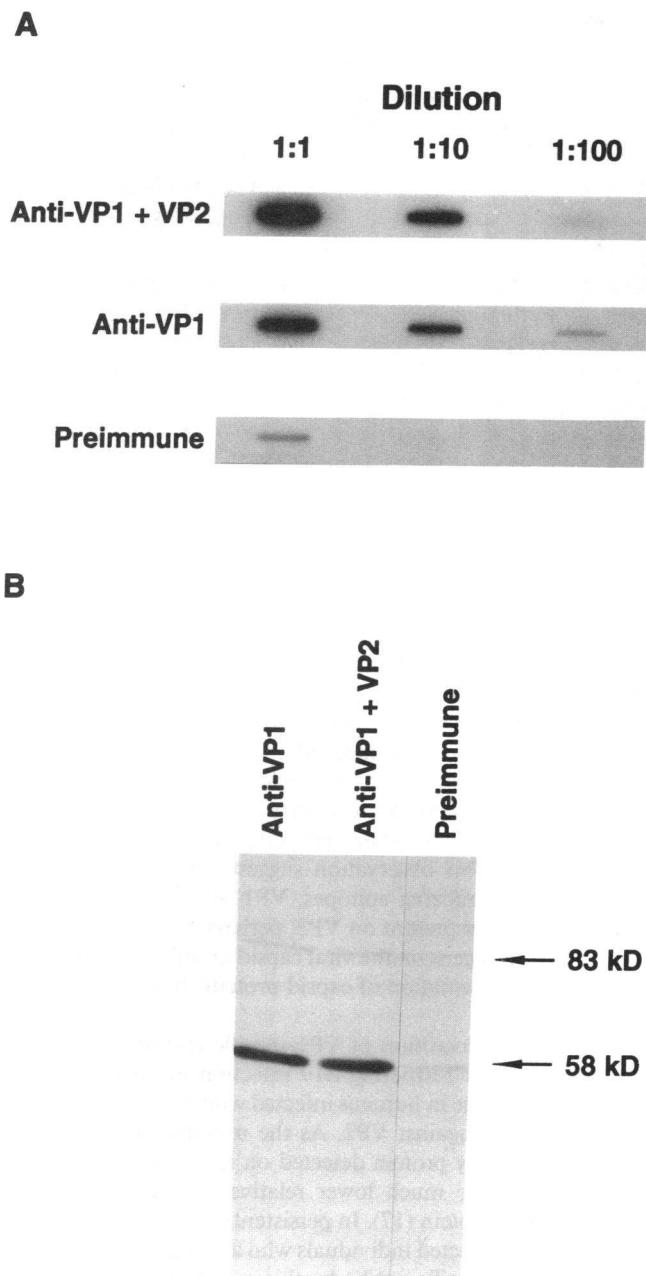


Figure 4. Immunoprecipitation of virions and empty capsids. (A) A pool of antisera raised against the VP1-specific fusion protein (anti-VP1) and antiserum raised against empty capsids containing both VP1 and VP2 (anti-VP1 + VP2) were used to immunoprecipitate virions from viremic human serum. Precipitated virus was detected using a B19-specific DNA probe. (B) The same antisera were used to immunoprecipitate labeled empty capsids produced in a baculovirus expression system. The positions of the major (58 kD) and minor (83 kD) capsid proteins are indicated.

ity at the maximal tested dilution of 1/250. These titers are comparable to those observed in convalescent human sera (8).

Discussion

Parvoviruses are among the simplest of viral particles. The icosahedral capsids are made up of 60 copies of the capsid proteins

VP1 and VP2. The structural unit of these proteins is an identical eight-stranded anti-parallel beta barrel homologous to similar structures in many other icosahedral viruses (6). VP1 and VP2 differ in the presence on the former of a unique amino-terminal domain. The structure of canine parvovirus has recently been solved to the atomic level by x-ray crystallography, and the position of VP2 in this virus is now known exactly (6). Canine parvovirus has a capsid protein distribution similar to B19, with the majority of the capsid made up of VP2 (or its derivative, VP3). The VP1 tail and the amino terminal 37 amino acids of VP2 are not detected by crystallographic techniques, probably because their position on the virion is not symmetric and is insufficiently constrained. Residue 38 of VP2 lies within the capsid, beneath a hollow cylinder on the fivefold axis, raising the possibility that the end of VP2 and perhaps the VP1 tail may extend through this cylinder to the outside of the virus. In support of this hypothesis, an ill-defined electron density was reported within the cylinder in virions that was absent in empty capsids, suggesting that the incorporation of DNA was associated with entry of the ends of the capsid proteins into the cylinders (6). Symmetry considerations (7) and the assumption that the basic residues on VP1 help stabilize the viral DNA led to the prediction that VP1 folds inside the viral capsid, and only the amino terminus of VP2 would enter the cylinders. We (17), and later others (18), have demonstrated that specific reactivity to the VP1 unique region develops during natural infection and that VP1 is the dominant species recognized by convalescent human serum (17). This reactivity might be a response to disrupted or incomplete virions. By assaying reactivity against intact particles, we have demonstrated here that the VP1 tail is exposed in naturally occurring virions as well as recombinant empty capsids.

In CPV, because only full virions had an electron density within the core of the cylinder on the fivefold axis, it was inferred that neither VP2 nor the VP1 tail extends outside the protein shell in naturally occurring empty capsids. In contrast, we have shown that the VP1 tail is external in recombinant B19 parvovirus empty capsids. There are many possible explanations for this discrepancy. First, CPV VP1 may be both structurally and functionally different from B19 VP1. The unique VP1 region of CPV is only 153 amino acids long, in contrast to 226 amino acids in B19. Unlike CPV and other parvoviruses, the amino terminus of B19 VP1 is not particularly basic, making a DNA-binding role unlikely. Alternatively, while the cylinder on the CPV fivefold axis provides an attractive and appropriately placed site for the amino terminus of VP2 to pass through the protein shell, the data to support this role are indirect. There are no crystallographic data to localize the amino terminus of VP1.

If the tail of VP1 is outside the virion capsid, what is its function? Its external position and structural properties may offer some clues. There is an extended region beginning 12 amino acids from the amino terminus that has the potential to form an amphipathic helix. External amphipathic helices have been reported on picornaviruses (19), and they may represent regions where the virion interacts with the host cell membrane. The mechanism by which B19 and other unenveloped animal viruses traverse the lipid bilayer of the cell membrane or the endosome is unknown, but at some stage a membrane-binding protein must play a role, for which VP1 is a reasonable candidate. Another possibility is that VP1 is the ligand for the viral receptor. However, host range mutants of canine and feline

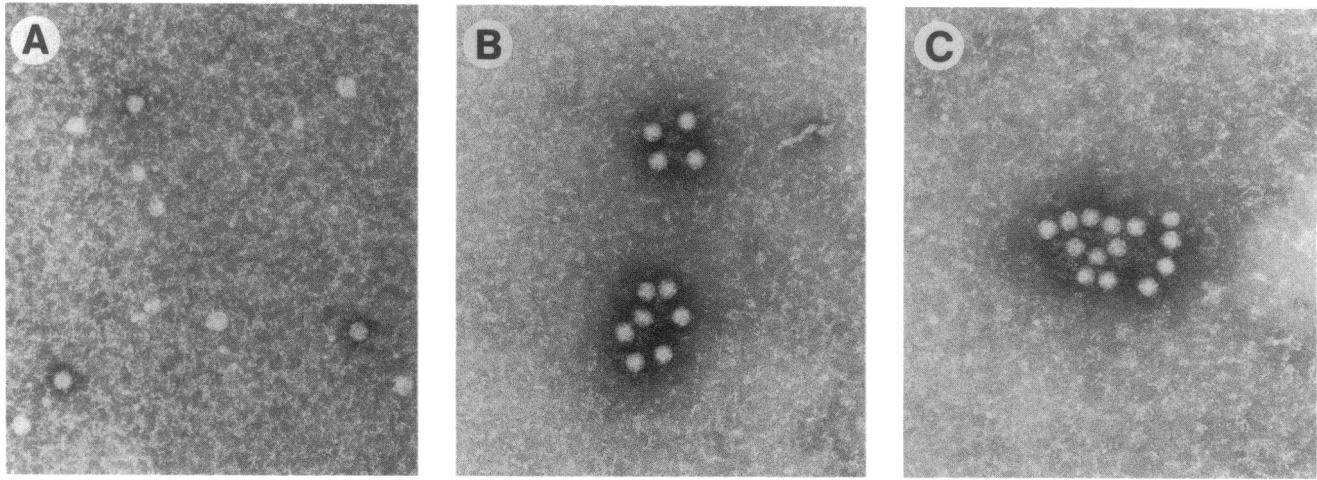


Figure 5. Immune electron microscopy of B19 parvovirus particles from infectious human serum. (A) Preimmune rabbit serum: virus particles were not agglutinated or antibody coated. (B and C) Immune rabbit sera from rabbit 1 (B) and rabbit 4 (C) agglutinated virus particles and coated them with antibody. Two additional rabbit antisera were not tested. Negative stains, $\times 171,000$.

parvoviruses have been mapped to regions of VP2 (20), making this a more likely area for receptor binding in these structurally similar viruses. Either as a membrane contact region or receptor ligand, VP1 would be expected to be an efficient target for neutralizing antibody.

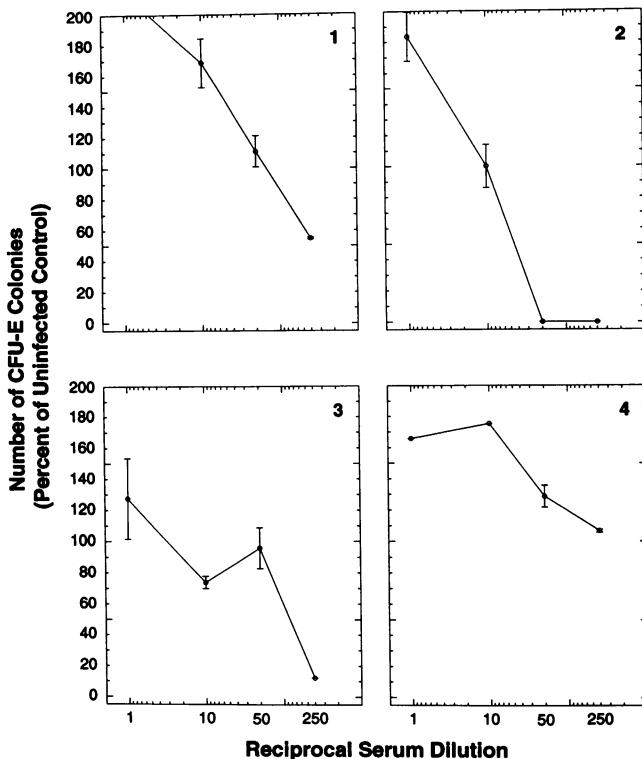


Figure 6. Neutralization activity of anti-VP1 antiserum. Neutralization activity was assessed by measuring the ability of antiserum to restore CFU-E colony formation in fresh cultures of bone marrow mononuclear cells exposed to B19. All colony numbers shown are the mean of two experiments \pm standard deviation and are expressed as percentage of uninfected controls. Each panel represents the neutralization data from a single rabbit (rabbits 1 through 4, respectively).

External localization of the VP1 tail only partially explains the requirement for VP1 to be present to generate neutralizing antisera. While VP1 clearly does contain neutralizing epitopes, we and others have identified other neutralizing epitopes on VP2 (9–11). Neutralizing monoclonal antibodies directed against these VP2 epitopes recognize empty capsids containing only VP2, but have only been generated using intact virions as immunogens. This observation suggests that, in addition to containing neutralizing epitopes, VP1 modulates the antigenicity of epitopes present on VP2, perhaps by exposing otherwise cryptic antigens on the viral capsid or influencing the processing and presentation of capsid proteins by cells of the immune system.

Humoral recognition of VP1-specific epitopes appears to be important for restricting B19 infection *in vivo*. The early antibody response in humans infected with the virus is directed almost entirely against VP2. As the response matures, however, the primary protein detected on immunoblots becomes VP1, despite the much lower relative concentration of the minor capsid protein (17). In persistently infected patients, including HIV-infected individuals who are able to generate high levels of B19-specific antibody, this switch from VP2 to VP1 reactivity does not occur. VP1 is also the major specificity of pooled human immunoglobulin (17), used in the treatment of chronic infection. Thus, responsiveness to VP1 correlates with *in vivo* activity against the virus.

The external location of the VP1 tail in empty capsids provides a unique site for engineering recombinant vaccines and protein delivery systems. While some portion of the VP1 protein must pass through the protein shell, our data suggest that the majority is external, unconstrained, and antigenic. The external region might be replaced by an unrelated protein to present antigen to the immune system in a particulate context. This system has several potential advantages over other recombinant virus platforms. Empty capsids are fundamentally replication incompetent. They can be produced in systems that are free of other viral proteins or nucleic acids, making recombination or the accidental encapsidation of pathogenic genes improbable. Unlike poliovirus vectors, where neutralizing epitopes representing very limited portions of external protein

loops can be replaced with foreign oligopeptides (21–23), the external domain of VP1 may be able to accommodate large heterologous protein domains, allowing the presentation of multiple antigenic sites or the delivery of enzymatic activity. If self-assembly can occur with heterologous proteins replacing the tail of VP1, a baculovirus production system would provide a ready source of easily purified and safe antigen for vaccine development.

Acknowledgments

Human serum containing a high titer of infectious virus was the generous gift of Bernard Cohen, Virus Reference Laboratory, Central Public Health Laboratory, Colindale, London, England.

References

1. Young, N. 1988. Hematologic and hematopoietic consequences of B19 parvovirus infection. *Semin. Hematol.* 25:159–172.
2. Ozawa, K., G. Kurtzman, and N. Young. 1987. Productive infection by B19 parvovirus of human erythroid bone marrow cells in vitro. *Blood.* 70:384–391.
3. Frickhofen, N., J. L. Abkowitz, M. Safford, J. M. Berry, J. Antunez-de-Mayolo, A. Astrow, R. Cohen, I. Halperin, L. King, and D. Mintzer. 1990. Persistent B19 parvovirus infection in patients infected with human immunodeficiency virus type 1 (HIV-1): a treatable cause of anemia in AIDS. *Ann. Intern. Med.* 113:926–933.
4. Anderson, L. J., and E. S. Hurwitz. 1988. Human parvovirus B19 and pregnancy. *Clin. Perinatol.* 15:273–286.
5. Siegl, G. 1988. Patterns of parvovirus disease in animals. In *Parvoviruses and Human Disease*. J. R. Pattison, editor. CRC Press, Boca Raton, FL. 43–67.
6. Tsao, J., M. S. Chapman, M. Agbandje, W. Keller, K. Smith, H. Wu, M. Luo, T. J. Smith, M. G. Rossman, and R. W. Compans. 1991. The three-dimensional structure of canine parvovirus and its functional implications. *Science (Wash. DC)*. 251:1456–1464.
7. Carter, B. J., and P. J. Tattersall. 1991. Parvoviridae. In *Animal Virus Structure*. M. V. Nermut and A. C. Stevens, editors. Elsevier Science Publishing Co., Inc., New York. 325–334.
8. Kajigaya, S., H. Fujii, A. M. Field, S. Anderson, S. Rosenfeld, L. J. Anderson, T. Shimada, and N. S. Young. 1991. Self-assembled B19 parvovirus capsids, produced in a baculovirus system, are antigenically and immunogenically similar to native virions. *Proc. Natl. Acad. Sci. USA.* 88:4646–4650.
9. Sato, H., J. Hirata, M. Furukawa, N. Kuroda, H. Shiraki, Y. Maeda, and K. Okochi. 1991. Identification of the region including the epitope for a monoclonal antibody which can neutralize human parvovirus B19. *J. Virol.* 65:1667–1672.
10. Yoshimoto, K., S. Rosenfeld, D. Kennedy, N. Frickhofen, S. Kajigaya, and N. S. Young. 1991. A second neutralizing epitope of B19 human parvovirus implicates the spike region in the immune response. *J. Virol.* 65:7056–7060.
11. Sato, H., J. Hirata, N. Kuroda, H. Shiraki, Y. Maeda, and K. Okochi. 1991. Identification and mapping of neutralizing epitopes of human parvovirus B19 by using human antibodies. *J. Virol.* 65:5485–5490.
12. Shade, R. O., M. C. Blundell, S. F. Cotmore, P. Tattersall, and C. R. Astell. 1986. Nucleotide sequence and genome organization of human parvovirus B19 isolated from the serum of a child during aplastic crisis. *J. Virol.* 58:921–936.
13. Keegan, K., and M. S. Collett. 1986. Use of bacterial expression cloning to define the amino acid sequences of antigenic determinants on the G2 glycoprotein of Rift Valley fever virus. *J. Virol.* 58:263–270.
14. Maniatis, T., E. R. Fritsch, and J. Sambrook. 1982. *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. 545 pp.
15. Collett, M. S., R. Larson, S. K. Belzer, and E. Retzel. 1988. Proteins encoded by bovine viral diarrhea virus: the genomic organization of a pestivirus. *Virology.* 165:200–208.
16. Ozawa, K., G. Kurtzman, and N. Young. 1986. Replication of the B19 parvovirus in human bone marrow cell cultures. *Science (Wash. DC)*. 233:883–886.
17. Kurtzman, G. J., B. J. Cohen, A. M. Field, R. Oseas, R. M. Blaese, and N. S. Young. 1989. Immune response to B19 parvovirus and an antibody defect in persistent viral infection. *J. Clin. Invest.* 84:1114–1123.
18. Rayment, F. B., E. Crosdale, D. J. Morris, J. R. Pattison, P. Talbot, and J. J. Clare. 1990. The production of human parvovirus capsid proteins in *Escherichia coli* and their potential as diagnostic antigens. *J. Gen. Virol.* 71:2665–2672.
19. Fricks, C. E., and J. M. Hogle. 1990. Cell-induced conformational change in poliovirus: externalization of the amino terminus. *J. Virol.* 64:1934–1945.
20. Parrish, C. R. 1991. Mapping specific functions in the capsid structure of canine parvovirus and feline panleukopenia virus using infectious plasmid clones. *Virology.* 183:195–205.
21. Sverdlov, E. D., S. A. Tsarev, S. V. Markova, V. M. Rostapshov, T. L. Azhikina, I. P. Chernov, A. E. Gorbalenya, M. S. Kolesnikova, L. I. Romanova, and N. L. Teterina. 1989. Insertion of short hepatitis virus A amino acid sequences into poliovirus antigenic determinants results in viable progeny. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 257:354–356.
22. Burke, K. L., D. J. Evans, O. Jenkins, J. Meredith, E. D. D'Souza, and J. W. Almond. 1989. A cassette vector for the construction of antigen chimaeras of poliovirus. *J. Gen. Virol.* 70:2475–2479.
23. Evans, D. J., J. McKeating, J. M. Meredith, K. L. Burke, K. Katrak, A. John, M. Ferguson, P. D. Minor, R. A. Weiss, and J. W. Almond. 1989. An engineered poliovirus chimaera elicits broadly reactive HIV-1 neutralizing antibodies. *Nature (Lond.)*. 339:385–8, 340.