JOURNAL OF VIROLOGY, June 1993, p. 3004–3009
0022-538X/93/063004-06$02.00/0

Vol. 67, No. 6

Neutralizing Linear Epitopes of B19 Parvovirus Cluster in the VP1 Unique and VP1-VP2 Junction Regions

TOMOKO SAIKAWA, STACIE ANDERSON, MIKIO MOMOEDA, SACHIKO KAJIGAYA,* AND NEAL S. YOUNG

Hematology Branch, National Heart, Lung, and Blood Institute, Bethesda, Maryland 20892

Received 15 September 1992/Accepted 22 February 1993

Presentation of linear epitopes of the B19 parvovirus capsid proteins as peptides might be a useful vaccine strategy. We produced overlapping fusion proteins to span the viral capsid sequence, inoculated rabbits, and determined whether the resulting antisera contained antibodies that neutralized the ability of the virus to infect human erythroid progenitor cells. Antibodies that bound to virus in an enzyme-linked immunosorbent assay were present in antisera raised against 10 of 11 peptides; strongest activity was found for antisera against the carboxyl-terminal half of the major capsid protein. However, strong neutralizing activity was elicited in animals immunized with peptides from the amino-terminal portion of the unique region of the minor capsid protein and peptides containing the sequence of the junction region between the minor and major capsid proteins. The development of neutralizing activity in animals was elicited most rapidly with the fusion peptide from the first quarter of the unique region. A 20-amino-acid region of the unique region of the minor capsid protein was shown to contain a neutralizing epitope. Multiple antigenic peptides, based on the sequence of the unique region and produced by covalent linkage through a polylysine backbone, elicited strong neutralizing antibody responses. Synthetic peptides and fusion proteins containing small regions of the unique portion of the minor capsid protein might be useful as immunogens in a human vaccine against B19 parvovirus.

B19 parvovirus is a common human pathogen (25). Although fifth disease in children, a rash illness with fever, is not itself a serious infection (2), acute parvovirus infection in adults, which can present as arthralgia and arthritis, can cause significant morbidity in young women as a result of chronic joint symptoms (8). Parvovirus infection of persons with underlying hemolysis, as in sickle cell anemia and hereditary spherocytosis, causes a severe anemia (24). Parvovirus infection can become persistent in patients with underlying immunodeficiency states (7). Although patients with AIDS and persistent parvovirus infection can be treated remediable with immunoglobulin infusions, expensive and frequent retreatment is required to control anemia. Infection of the midtrimester fetus after a maternal bout of fifth disease can result in hydrops fetalis, a fatal syndrome in the absence of intrauterine transfusion of erythrocytes (1, 15). B19 parvovirus infection has been serologically linked to other hematologic syndromes, including idiopathic thrombocytopenic purpura, agranulocytosis (14), and congenital pure erythrocyte aplasia (3a). Because of the limited availability of specific antibody testing, the full spectrum of parvovirus disease in humans is probably not known.

Studies of patients with persistent parvovirus infection have indicated that the humoral immune response to this virus is dominant. In infected volunteers, virus disappears from the circulation concurrent with the detection of specific immunoglobulin M (IgM) and IgG antibodies (3). Virus cytotoxicity for erythroid progenitor cells, the target of virus infection in humans, can be completely neutralized by addition of convalescent-phase serum to cultures (10, 13). Patients susceptible to chronic infection lack antibodies that recognize viral capsid proteins on immunoblots, and their sera fail to neutralize virus infectivity for bone marrow cells (10). Anemia due to persistent infection can be treated by infusion of normal human immunoglobulins, which contain neutralizing antibodies to parvovirus (6, 11).

Because B19 parvovirus does not propagate well in conventional cell culture, efforts to produce a vaccine reagent have focused on recombinant antigen strategies. After producing empty parvovirus capsids in a baculovirus system, we discovered that a small variation in the capsid structure had a profound influence on the immune response of inoculated animals. The parvovirus capsid contains two proteins, the major capsid protein, or VP2, and the minor capsid protein, VP1. These two proteins are derived from overlapping reading frames, and VP1 is identical to VP2 except that it contains an additional 227 amino acids at the amino terminus. Rabbits injected with VP2-only capsids make antibodies to virus, but these antisera fail to neutralize virus activity. In contrast, animals injected with VP1 plus VP2 capsids make neutralizing antisera comparable in titer to convalescent-phase human sera (9). In further experiments, we have shown that the unique region of VP1 is external to the capsid, as antisera raised to a fusion protein containing this sequence can immunoprecipitate virions and empty capsids (18). These antisera also neutralized virus activity, a finding of probable biological importance, since the dominant antibody species in normal immune serum is directed against VP1 (10). These results suggested the possibility that linear epitopes, presented as short peptide sequences, might be useful as vaccine reagents. We undertook the current study to systematically map the capsid sequence for such linear neutralizing epitopes.

MATERIALS AND METHODS

Antigens and antisera. Eleven fusion proteins that combined regions of B19 parvovirus structural protein and maltose-binding protein were prepared (23) and designated VPF1 to VPF11 (Fig. 1A). The B19 parvovirus-specific region ranged from 56 to 111 amino acids. Multiple antigenic

* Corresponding author.

peptides corresponding to amino acids of the unique region of VP1 were prepared by using covalent linkage to a polylysine core structure by Research Genetics (South Huntsville, Ala.) (21) (Table 1). Rabbits were immunized with 200 μg of fusion protein or 500 μg of peptide by subcutaneous inoculation of footpads, initially with antigen emulsified in complete Freund's adjuvant followed by injection at 2-week intervals with similar quantities of antigen in incomplete Freund's adjuvant.

ELISA. Antibody titer was tested by enzyme-linked immunosorbent assay ELISA. The preparation of recombinant empty capsids containing VP1 and VP2 has been described previously (9). One microgram of recombinant empty parvovirus capsid was attached to Immulon II microtiter plates (Dynatech, Chantilly, Va.) by overnight incubation in phosphate-buffered saline (PBS) at 4°C. After blocking with PBS containing 3% bovine serum albumin, the plates were washed with PBS containing 0.05% Tween 20. One hundred microliters of diluted antiserum was added to each well, and the wells were incubated for 1 h at room temperature. Plates were washed three times in PBS and then in 100 μl of peroxidase-conjugated goat anti-rabbit IgG (GIBCO BRL Life Technologies, Gaithersburg, Md.) for 1 h at room temperature. After a second wash in PBS, 200 μl of 0.1 M citrate buffer containing *o*-phenylenediamine and H_2O_2 was added to each well. The enzyme reaction was stopped with 4 N H_2SO_4, and the A_{490} was measured with an automated microplate reader (Bio-Rad Laboratories, Richmond, Calif.).

ELISA results are reported as the measured A_{490}. Absorbance of a concurrent saline control was usually about 0.05 to 0.06 U. Nonspecific binding for antigen or antiserum alone was determined separately. For absent antigen, substituted by 3% albumin, binding averaged 0.062 ± 0.005 (range, 0.054 to 0.075). Nonspecific binding of antisera was determined by the absorbance of each preimmune antiserum; for such antisera diluted 1:1,600, binding averaged 0.021 ± 0.013 (range, 0.005 to 0.049), and for antisera diluted 1:100, binding averaged 0.059 ± 0.047 (range, 0.006 to 0.177).

Immunoblotting. Reactivities of antisera were assayed by immunoblotting, using the materials and directions provided with the Protein Image Kit (United States Biochemical, Cleveland, Ohio). Empty capsids containing VP1 and VP2 were boiled in sample loading buffer for 3 min, electrophoresed through 8% polyacrylamide in sodium dodecyl sulfate, and transferred to polyvinylidene difluoride membranes in a transfer buffer containing 25 mM Tris-HCl and 192 mM glycine at 100 mA for 16 h. Membranes were sequentially treated with 1% blocking solution, incubated with antisera diluted 1:300 in PBS for 1 h at room temperature, and incubated with alkaline phosphatase-conjugated goat anti-rabbit IgG (GIBCO BRL Life Technologies) for 1 h at room temperature. After completion of the antibody incubations, membranes were soaked in substrate buffer consisting of 100 mM Tris-HCl (pH 9.5), 100 mM NaCl, and 50 mM $MgCl_2$; bound antibody was detected with a substrate solution containing nitroblue tetrazolium and 5-bromo-4-chloro-3-indolylphosphate *p*-toluidine salt (GIBCO BRL Life Technologies).

Erythroid colony assay. Neutralizing activity was determined by using erythroid progenitor assays of human bone marrow (13). Antibody-containing serum was heated to 56°C for 30 min to inactivate complement. Thirty or 90 μl of test serum was mixed with 30 μl of human serum containing infectious B19 parvovirus, diluted 1:10 or 1:30, and incubated for 2 h at 4°C. Bone marrow was harvested from

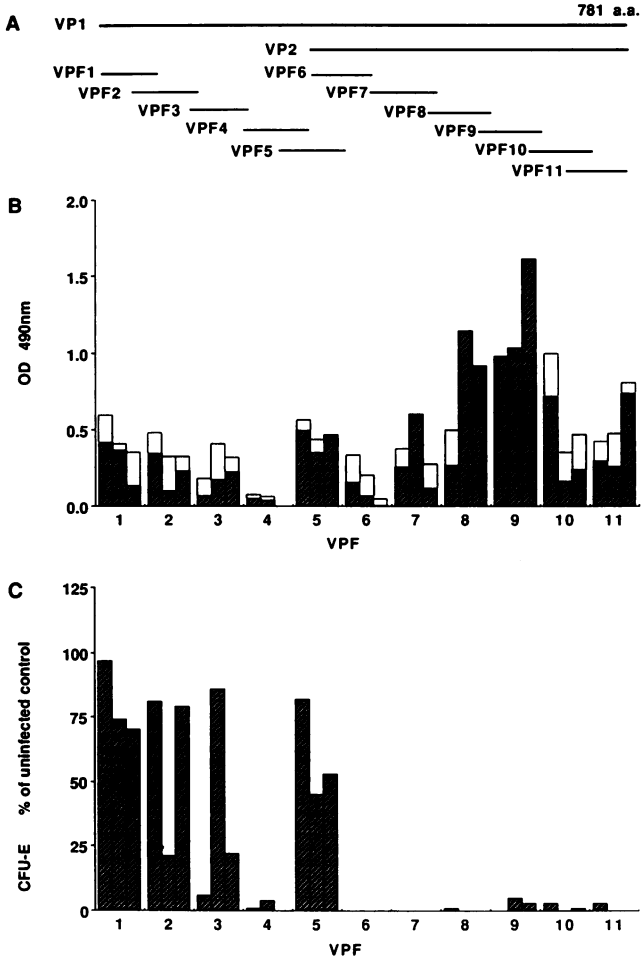

FIG. 1. Comparison of binding and neutralizing activity in antisera obtained after injection of rabbits with fusion peptides spanning the B19 parvovirus capsid protein sequence. (A) Locations of fusion protein sequences relative to the minor capsid protein sequence. Amino acids (a.a.) spanned by the proteins: VPF1, 1 to 94; VPF2, 52 to 142; VPF3, 122 to 177; VPF4, 158 to 227; VPF5, 187 to 265; VPF6, 228 to 334; VPF7, 314 to 423; VPF8, 403 to 513; VPF9, 493 to 603; VPF10, 583 to 693; VPF11, 673 to 781. (B) Binding activity on ELISA of antisera raised to fusion proteins. Three rabbits were inoculated for each VPF protein except for VPF4, for which two rabbits were inoculated. ELISA was performed with recombinant empty capsids containing VP1 and VP2 as antigens (VP1 constituted about 10% of protein in the capsids). Assays of sera obtained at 12 weeks are shown. Shown in dark bars are results of a typical experiment (one of two performed) with all sera diluted 1:1,600, a concentration that resulted in linear spectrophotometric optical density (OD) readings in this assay for the highest-binding-titer sera. Shown in lighter extensions of each bar are results when binding was assayed at a higher concentration of each serum (1:100), in which binding was not linear for the high-titer sera. (C) Neutralizing activity of antisera. The 12-week antisera used for panel B were tested for the ability to protect human erythroid progenitor cells against B19 parvovirus cytotoxicity in a marrow colony assay. One of two similar experiments is illustrated. In this experiment, 77 CFU-E were measured per 10^5 cells; data are expressed as percentage of the value for uninfected control plates.

TABLE 1. Amino acids sequences of short fragments of VPF1

Fragment	No. of amino acids	Sequence
MAP-F1	20	MSKKSGKWWESDDKFAKAVY
MAP-F2	20	AKAVYQQFVEFYEKVTGTDL
MAP-F3	21	TGTDLELIQILKDHYNISLDN

normal donors directly into syringes containing preservative-free heparin and Iscove's modification of Dulbecco's medium, under a protocol approved by Institutional Review Board of the National Heart, Lung, and Blood Institute. Mononuclear cells were isolated over a Ficoll-Hypaque gradient. Cells were incubated with the virus-serum mixture at a final concentration of 3×10^6 cells per ml for 1 h at 4°C to allow virus adsorption and then diluted in standard tissue culture medium for late erythroid progenitor cells (CFU-E) containing 0.8% methylcellulose, 30% fetal calf serum, 1% bovine serum albumin, 10 mM β-mercaptoethanol, and 10 U of recombinant erythropoietin (Amgen Biologicals, Thousand Oaks, Calif.) per ml. Cultures were incubated for 7 to 8 days at 37°C in 95% humidity and 5% CO_2, and colonies were counted visually. All progenitor assays were performed in duplicate; controls included virus alone, virus with known neutralizing serum, and cells without either virus or serum.

Immunoglobulin purification and concentration. To concentrate rabbit IgG for some experiments, 15 to 20 ml of antiserum, diluted 1:1 in buffer (ImmunoPure IgG binding buffer; Pierce, Rockford, Ill.), was applied to a column of immobilized protein A (Affinity Pak Column; Pierce). The column was washed in 10 ml of binding buffer, and the IgG was eluted with 10 ml of ImmunoPure IgG elution buffer (Pierce). One-milliliter fractions were collected, and the fractions with highest A_{280} (usually the second, third, and fourth milliliters) were pooled. Purified IgG was dialyzed overnight in PBS and then concentrated in an Amicon Centricon 30 (W. R. Grace, Beverly, Mass.).

RESULTS

Production of reactive antisera. Rabbits were immunized with fusion peptides, and sera obtained at 12 weeks were tested for reactivity to parvovirus capsid proteins by immunoblotting. Antisera against VP1 unique region sequences, produced by inoculation with fusion peptides VPF1 to VPF4, reacted exclusively with VP1 capsid protein; antisera raised to peptide sequences common to VP1 and VP2, VPF5 to VPF11, reacted to both capsid proteins on Western immunoblot analysis (Fig. 2). All antisera also showed strong reactivity on immunoblots to the peptides used as antigens (data not shown).

Binding of anti-fusion peptide antisera to empty capsids. Antisera were then tested in an ELISA for reactivity against empty parvovirus capsids. Reactivity was determined at a variety of dilutions for each sera. Antisera were tested at a dilution of 1:1,600 (Fig. 1B, dark bars), previously shown to be in the linear range for the assay, and at a higher concentration (Fig. 1B, 1:100, light extensions of dark bars), saturating in this assay for higher-titer sera. At the lower concentration, binding of antisera was not uniform for the entire capsid sequence and was most prominent for VPF8 and VPF9 and absent for other regions, including VPF4 and VPF6. At higher concentrations of sera, binding activity increased for VPF6 and for other antisera that gave intermediate reactivity on initial testing at a greater dilution.

Localization of neutralizing regions. The ability of antisera to neutralize virus activity did not parallel binding activity (Fig. 1C). Antisera raised against peptides from the carboxyl-terminal half of VP2, which showed the greatest binding activity, were ineffectual in protecting erythroid progenitor cells against viral toxicity. In contrast, antisera directed against the unique region of VP1 and the junction region between VP1 and VP2 were highly protective in the colony assay.

Because the region contained within VPF6 includes a neutralizing epitope recognized by a monoclonal antibody raised to intact virions, we were surprised by the absence of neutralizing activity of these antisera, even when added at threefold-higher amounts to the cultures (Fig. 3B). However, when we purified the antisera by affinity chromatography and tested these highly concentrated immunoglobulin preparations in the colony assay, significant neutralizing activity was detected for the antisera produced against VPF4, VPF6, VPF9, and VPF10 (Fig. 3C). In contrast, purified immunoglobulin from antisera raised to VPF7, VPF8, and VPF11 showed little reactivity after fractionation, even when assayed against lower virus concentrations (Fig. 3C).

Further analysis of antisera produced against VPF1 to VPF4 suggested that the most amino-terminal segment of the unique region was most immunogenic. Sera from phlebotomies at 6, 8, and 12 weeks after the initial inoculum were compared (Fig. 4). Neutralizing activity was apparent in antisera to VPF1 at the time of the first bleed; neutralizing activity appeared in the blood of animals injected with VPF2 and VPF3 at 8 weeks but increased further at 12 weeks; neutralizing activity could not be detected in the sera of animals receiving VPF4. Concentration-purified immuno-

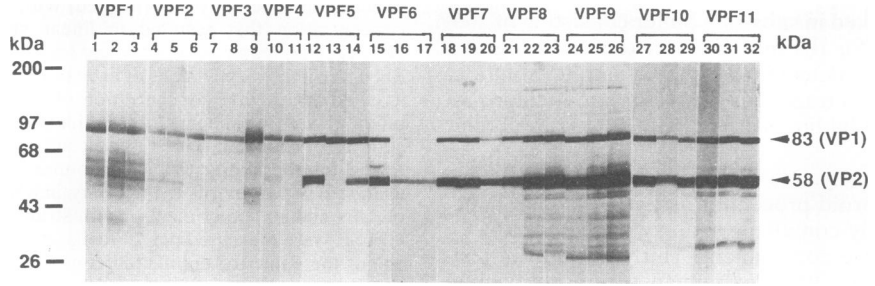

FIG. 2. Immunoblot analysis of antisera raised to VPF1 to VPF11 tested against parvoviral capsid proteins electrophoresed under denaturing conditions in 8% polyacrylamide.

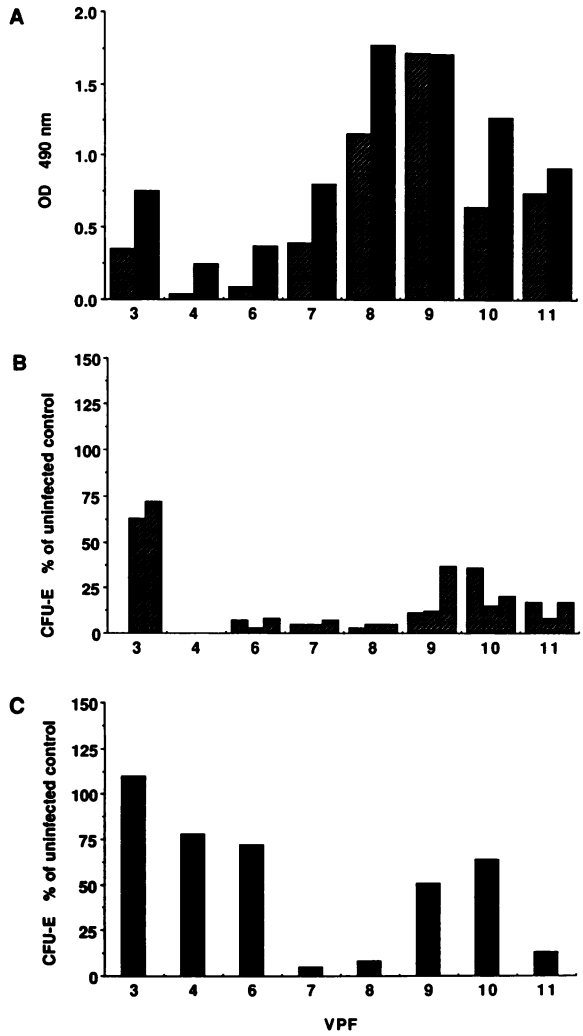

FIG. 3. Neutralizing activity of purified and concentrated antisera against parvovirus fusion proteins that showed low reactivity in initial assays. (A) ELISA reactivity of prepurified pooled antisera (▨) compared with purified antibody, also obtained from pooled sera (■). OD, optical density. (B) Neutralizing activity of antisera added at threefold-higher amounts to marrow cultures. Each bar represents results from a single rabbit serum. (C) Neutralizating activity of purified immunoglobulin, obtained from pooled sera. Antisera were diluted 1:1,000 for the experiment shown in panel A. Virus was used at higher (1:10 dilution of stock-infected serum; B) and lower (1:30 dilution; C) concentrations to detect low levels of neutralizing activity. In each case, one of two similar experiments is shown. In the experiments shown in panels B and C, uninfected marrow contained 60 and 41 CFU-E per 10^5 cells, respectively; results are expressed as percentage of the value for uninfected control plates.

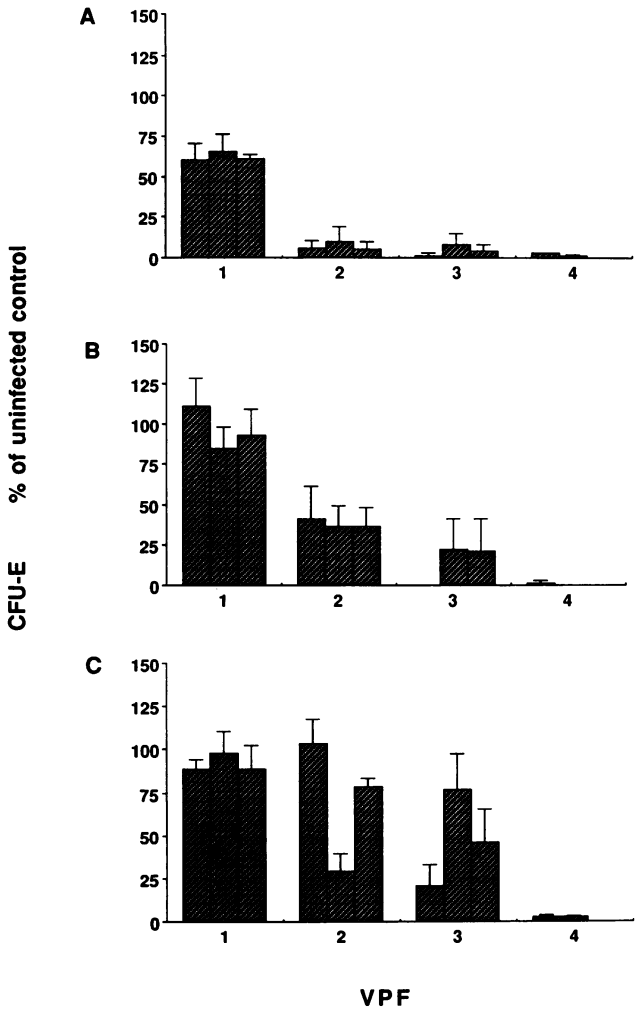

FIG. 4. Comparison of neutralizing activity of antisera obtained 6 (A), 8 (B), and 12 (C) weeks after initial inoculation of rabbits with VPF1 to VPF4. Animals were inoculated at days 1, 14, 28, 42, 56, and 70. Results are means ± standard errors of the means of three experiments. Data are expressed as percentage of the value for uninfected control plates.

globulin from the antisera to VPF4 did reveal some neutralizing activity (Fig. 3C).

Neutralizing antisera elicited with synthetic peptides. Multiple antigenic peptides were synthesized to correspond to sequences within the unique region of VP1; these sequences were selected because they had been shown to elicit neutralizing antibodies when presented as fusion proteins. These three peptides, termed MAP-F1 (corresponding to VP1 amino acids 1 to 20), MAP-F2 (16 to 35), and MAP-F3 (31 to 51), spanned the most amino-terminal portion of the unique region of VPF1 but did not overlap with VPF2. When used as immunogens in rabbits, some peptides were able to elicit neutralizing antibodies (Fig. 5). Two of two rabbits injected with MAP-F3 produced high-titer neutralizing antisera; both antisera also bound to fusion protein VPF1 on immunoblots (data not shown). One of two rabbits immunized with MAP-F2 produced neutralizing antiserum; this animal's serum, after concentration, showed binding on immunoblots to VP1 but not to fusion protein VPF1, and the second animal immunized with MAP-F2 had no evidence of either binding or neutralizing activity. For MAP-F1, neither animal produced neutralizing antisera, and both sera showed binding activity to VP1 but not to the fusion protein VPF1.

DISCUSSION

Our results demonstrate the feasibility of a peptide subunit vaccine for B19 parvovirus. Sequences from the amino-terminal portion of the capsid protein appear especially

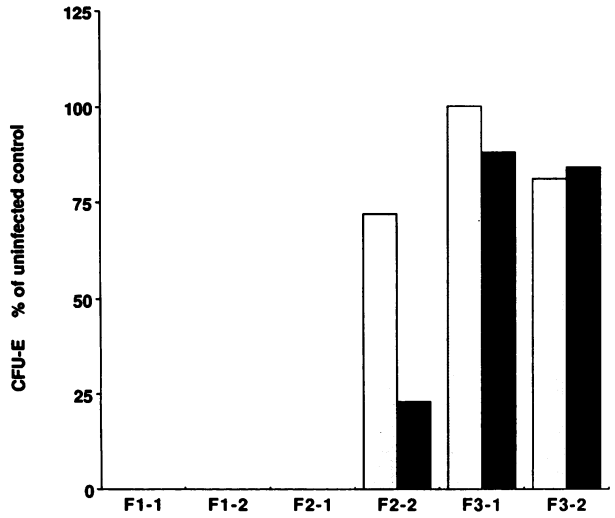

FIG. 5. Neutralizing activity of antisera raised against synthetic multiantigenic peptides. Animals were inoculated with synthetic antigens, and protective activity in the colony assay was determined for sera obtained at 8 (□) and 10 (■) weeks. One of two typical experiments is illustrated; CFU-E were equal to $43/10^5$ in control plates, and results are expressed as percentage of the value for uninfected control plates.

suitable for this purpose. As predicted from our earlier success in producing neutralizing antisera by inoculation of a fusion protein containing the entire unique region of VP1 (18), smaller segments of the unique region were also immunogenic and immunization resulted in neutralizing antisera. As judged from the rapidity with which neutralizing activity developed in animals, the most amino-terminal quarter of the unique region was most immunogenic and the most carboxyl-terminal quarter was least immunogenic. Somewhat surprisingly, the peptide that contained the fusion region between VP1 and VP2 was also highly immunogenic. Neutralizing antisera could also be elicited with synthetic peptides from the VP1 unique region, and one limited amino acid sequence was shown to contain a neutralizing epitope.

While most of the fusion peptides derived from the major capsid protein sequence were antigenic in rabbits, there was no correlation between binding capacity and neutralization activity. In comparison with peptides VPF1 to VPF3 from the VP1 unique region, VP2 peptides were much less effective in eliciting neutralizing activity in animals. Some peptides, in particular VPF7, VPF8, and VPF11, showed little or no ability to elicit neutralizing antibodies, and others, like VPF6, VPF9, and VPF10, invoked minimal responses, measurable only after antibody purification and concentration and after challenge by lower concentrations of virus. These results were all the more surprising because some of these relatively weak immunogenic peptides contain sequences known from other experiments to contain neutralizing epitopes. Some of these sequences would be predicted to be immunogenic by analogy with the detailed structure of canine parvovirus (22). Sato et al. (19) and Yoshimoto et al. (23) each found a monoclonal antibody, produced by the cells of an animal immunized with B19 virions, that neutralized virus activity in the CFU-E assay and recognized capsid protein on immunoblots. Specific binding to synthetic peptides was found for amino acids 328 to 344 of VP2 (19), a site predicted to lie in the GH loop of the dimple rim (5) and contained within VPF9, and amino acids 57 to 77 of VP2 (23), in loop 1 of the capsomere structure and contained within VPF6. Seropositive human plasma was found to contain antibodies that recognized a variety of B19 parvovirus capsid protein peptides, and after affinity purification, six antibodies neutralized virus activity (20); the peptide sequences were located mainly at the carboxyl terminus of VP2: amino acids 253 to 272 (loop 3, within VPF8), 309 to 330 and 325 to 345 (both from the GH loop and within VPF9), 359 to 382 (between the dimple and the canyon, within VPF10), 449 to 468 (canyon), and 491 to 515 (B1, both sequences within VPF11). Clearly these sequences, when presented as part of the capsid or in intact virions, are sufficiently immunogenic when presented to the murine or human immune system. While the antibodies retain the ability to recognize linear sequences of capsid protein, even large peptides do not reproduce the capsid structure when used as immunogens. That the conformation of the peptide sequence undergoes major conformational transformation when inserted into a capsid is also illustrated by the inability of VP2-only empty capsids to elicit a neutralizing antibody response, whereas VP1 plus VP2 capsids are highly immunogenic. As we suggest elsewhere, insertion of VP1 into the capsid structure may allow recognition of prominent loop structures about the major spike of the capsid; in contrast, antibodies appear to have access to β-pleated sheets in the more interior regions of the capsomere when capsids lack VP1 (5).

Why are the VP1 unique region and both fusion and synthetic peptides derived from this sequence so effective in eliciting neutralizing antibodies? While there is no definitive answer to this question, two appealing possibilities are that this region serves either as the binding site for the (unknown) cellular receptor of the virus or as a site of proteolytic cleavage crucial to virus decapsidation and delivery of the viral genome to the cell. The atomic fine structure of canine parvovirus is unhelpful, as neither the VP1 unique region of this virus nor the first 38 amino acids of VP2 were resolved by crystallography. Experimental evidence against the first hypothesis is the equivalency of the VP2-only capsids to capsids or virions containing VP1 in hemagglutination assays (4), assuming that hemagglutination is a fair reflection of physiologic binding. While there is no direct laboratory evidence for cleavage of a VP1 peptide after binding, sequence from the junction region between VP1 and VP2 was a potent immunogen despite its predicted location interior to the capsomere (22), suggesting the accessibility of this region in virions at some stage of infection. Parallel to our data, a junction fragment fusion peptide of canine parvovirus also has been described as uniquely capable of eliciting neutralization activity, whereas synthetic peptides from within the major capsid protein were ineffective (12). Extrapolation of antibody data to statements concerning parvovirus capsid structure is hazardous, as considerations of peptide solubility and conformation as well as experimental variability may be the most important parameters. Nonetheless, these results suggest that immune recognition of the unique portion of VP1 is similar to reactivity against a soluble, conformationally free globular protein, while immune recognition of VP2 epitopes is much more conformationally fixed by the capsomere structure.

Several synthetic peptides containing about 20 amino acids of the unique region of VP1 elicited neutralizing antisera in rabbits. While both B-cell (16) and T-cell (17) epitopes of canine parvovirus have been identified by using synthetic peptides, this is the first demonstration of the

ability to produce biological activity against a parvovirus with use of synthetic peptides. Synthetic peptides, perhaps also directed against VP1 unique regions of canine, feline, and porcine parvoviruses, offer an alternative to attenuated virus for protection of animals against disease. For humans, such peptides, alone or in combination with a recombinant capsid, may be convenient and safe primary or secondary (boosting) vaccine reagents.

REFERENCES

1. **Anderson, L. J., and E. S. Hurwitz.** 1988. Human parvovirus B19 and pregnancy. Clin. Perinatol. **15:**273–286.
2. **Anderson, M. J., and J. D. Cherry.** 1987. Parvoviruses, p. 1646–1653. *In* R. D. Feigin and J. D. Cherry (ed.), Textbook of pediatric infectious diseases. W. B. Saunders, Philadelphia.
3. **Anderson, M. J., P. G. Higgins, L. R. Davis, J. S. Willman, S. E. Jones, I. M. Kidd, J. R. Pattison, and D. A. J. Tyrrell.** 1985. Experimental parvoviral infection in humans. J. Infect. Dis. **152:**257–265.

3a. **Brown, K., J. Antunez-de-Mayolo, and N. S. Young.** Unpublished data.

4. **Brown, K. E., and B. J. Cohen.** 1992. Haemagglutination by parvovirus B19. J. Gen. Virol. **73:**2147–2149.
5. **Chapman, M. S., and M. G. Rossmann.** 1992. Structure, sequence, and function correlations among parvoviruses. Virology, in press.
6. **Frickhofen, N., J. Abkowitz, M. Safford, J. M. Berry, J. Antunez-de-Mayolo, A. Astrow, R. Cohen, I. Halperin, L. King, D. Mintzer, B. Cohen, and N. S. Young.** 1990. Persistent parvovirus infection in patients infected with human immunodeficiency virus-1: a treatable cause of anemia in AIDS. Ann. Intern. Med. **113:**926–933.
7. **Frickhofen, N., and N. S. Young.** 1989. Persistent parvovirus B19 infections in humans. Microb. Pathog. **7:**319–327.
8. **Joseph, P. R.** 1986. Fifth disease: the frequency of joint involvement in adults. N.Y. State Med. J. **86:**560–563.
9. **Kajigaya, S., H. Fujii, A. M. Field, 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.
10. **Kurtzman, G., R. Cohen, A. M. Field, R. Oseas, R. M. Blaese, and N. Young.** 1989. The immune response to B19 parvovirus infection and an antibody defect in persistent viral infection. J. Clin. Invest. **84:**1114–1123.
11. **Kurtzman, G., N. Frickhofen, J. Kimball, D. W. Jenkins, A. W. Nienhuis, and N. S. Young.** 1989. Pure red-cell aplasia of 10 years' duration due to persistent parvovirus B19 infection and its cure with immunoglobulin therapy. N. Engl. J. Med. **321:** 519–523.
12. **Lopez de Turiso, J., E. Cortes, A. Ranz, J. Garcia, A. Sanz, C. Vela, and J. I. Casal.** 1991. Fine mapping of canine parvovirus B cell epitopes. J. Gen. Virol. **72:**2445–2456.
13. **Mortimer, P. P., R. K. Humphries, J. G. Moore, R. H. Purcell, and N. S. Young.** 1983. A human parvovirus-like virus inhibits hematopoietic colony formation in vitro. Nature (London) **302:** 426–429.
14. **Pont, J., E. Puchhammer-Stöckl, A. Chott, T. Popow-Kraupp, H. Keinzer, G. Postner, and N. Honetz.** 1992. Recurrent granulocytic aplasia as clinical presentation of a persistent parvovirus B19 infection. Br. J. Haematol. **80:**160–165.
15. **Public Health Laboratory Service Working Party on Fifth Disease.** 1990. Prospective study of human parvovirus (B19) infection in pregnancy. Br. Med. J. **300:**1166–1170.
16. **Rimmelzwaan, G. F., J. Carlson, F. G. UytdeHaag, and A. D. Osterhaus.** 1990. A synthetic peptide derived from the amino acid sequence of canine parvovirus structural proteins which defines a B cell epitope and elicits antiviral antibody in BALB c mice. J. Gen. Virol. **71:**2741–2745.
17. **Rimmelzwaan, G. F., M. C. M. Poelen, R. H. Meleon, J. Carlson, F. G. C. M. UutdeHaag, and A. D. M. E. Osterhaus.** 1990. Delineation of canine parvovirus T cell epitopes with peripheral blood mononuclear cells and T cells clones from immunized dogs. J. Gen. Virol. **71:**2321–2329.
18. **Rosenfeld, S. R., K. Yoshimoto, S. Anderson, S. Kajigaya, N. S. Young, P. Warrener, G. Bonsal, and M. Collett.** 1992. The unique region of the minor capsid protein of human parvovirus B19 is exposed on the virion surface. J. Clin. Invest. **89:**2023–2029.
19. **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.
20. **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:**5845–5490.
21. **Tam, J. P.** 1988. Synthetic peptide vaccine design: synthesis and properties of a high-density multiple antigenic peptide system. Proc. Natl. Acad. Sci. USA **85:**5409–5413.
22. **Tsao, J., M. S. Chapman, M. Agbandje, W. Keller, K. Smith, H. Wu, M. Luo, T. J. Smith, M. G. Rossmann, R. W. Compans, and C. R. Parrish.** 1991. The three-dimensional structure of canine parvovirus and its functional implications. Science **251:**1456–1464.
23. **Yoshimoto, K., S. Rosenfeld, N. Frickhofen, D. Kennedy, S. Kajigaya, and N. S. Young.** 1991. A second neutralizing epitope of B19 parvovirus implicates the spike region in the immune response. J. Virol. **65:**7056–7060.
24. **Young, N.** 1988. Hematologic and hematopoietic consequences of B19 parvovirus infection. Semin. Hematol. **25:**159–172.
25. **Young, N. S.** 1992. Human (B19) parvovirus, N. S. Young (ed.), Viruses in hematology. Marcel Dekker, New York.