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Localization of an Immunodominant Domain on Baculovirus-Produced Parvovirus B19 Capsids: Correlation to a Major Surface Region on the Native Virus Particle

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An immunodominant region on baculovirus-produced parvovirus B19 VP2 capsids was localized between amino acids 259 and 426 by mapping the binding sites of a panel of monoclonal antibodies which recognize determinants on the particles. The binding sites of three monoclonal antibodies were fine-mapped within this antigenic domain. Six VP2-specific monoclonal antibodies recognized determinants common to both the empty capsids and native parvovirus. The defined antigenic region is most probably exposed on the native B19 virion and corresponds to part of the threefold spike on the surface of canine parvovirus particles.

Human parvovirus B19 causes the fifth disease of childhood, a mild infection, and a variety of other illnesses as a result of its almost exclusive replication in erythroid progenitor cells of the bone marrow (21, 38). In individuals with an increased requirement for erythrocytes in combination with hemolysis, parvovirus B19 causes transient aplastic crisis, and in immunocompromised patients, it can cause prolonged anemia (35, 37). Primary infection in pregnancy has been associated with spontaneous abortion and hydrops fetalis (1, 33). The requirement of B19 virus for erythroid progenitor cells has meant that culture of the virus has not been established in a cell line; to obtain antigen for diagnostic use and possibly a vaccine, expression systems have been used. Empty capsids consisting of the two capsid proteins VP1 and VP2 have been produced in Chinese hamster ovary cells (15) and insect cells (4, 14). Expression of VP1 and VP2 independently from two baculovirus recombinants showed that VP2 (the major capsid protein) can alone assemble into empty capsids in insect cells whereas VP1 cannot. The VP2 and VP1/VP2 capsids produced in insect cells are similar to native virus in size, appearance, and stoichiometry in the case of the capsids containing VP1 (4). VP1 differs from VP2 in that it has an N-terminal extension of 227 amino acids which is thought to be internally located (6, 9). In a capture enzyme-linked immunosorbent assay (ELISA) in which the reactivities of the capsids with B19 virus specific antibodies bound to the solid phase were tested and in which a degree of conformation of the capsids is retained, both types of insect cell-produced capsids were recognized by all human sera containing B19 virus-specific antibodies that were tested (23). For the development of a B19 virus vaccine based on the recombinant capsids, it will be necessary to analyze, among other properties, the immunogenicity and epitopes of the capsids, the latter in comparison with those mapped on the native virion. Since it may also be possible to use these recombinant B19 virus capsids as a vaccine carrier, as has been described for a number of polymeric particulate proteins (reviewed in reference 16), the mapping of epitopes may reveal candidate sites for the insertion of foreign epitopes. To achieve these aims, a panel of monoclonal antibodies (MAbs) was made by immunizing mice with the purified recombinant VP2 capsids. Their binding sites were mapped by using overlapping bacterial fusion proteins spanning the whole of the capsid protein VP1, and fine-mapping was carried out by means of an ELISA with overlapping synthetic nonapeptides (PEPSCAN) spanning the region to which most MAbs were shown to bind. Epitopes recognized by two MAbs raised against native B19 virus were also mapped.

The results presented here demonstrate an important, immunodominant region on the synthetic VP2 capsids which correlates with a similar region found on native B19 virions. Further, three determinants that may represent immunoexposed epitopes could be precisely defined.

MATERIALS AND METHODS

Expression of B19 virus capsid proteins in insect cells. Construction of the recombinant baculoviruses expressing VP1, VP2 (3), and both VP1 and VP2 (4) has been described previously. Baculoviruses were grown in *Spodoptera frugiperda* cells (5), obtained from the American Type Culture Collection (CRL 1711), in TC-100 medium (GIBCO/BRL) containing 10% fetal calf serum and 50 μg of gentamicin per ml. Infections were carried out on monolayers of *S. frugiperda* cells at a multiplicity of infection of 1 PFU per cell, and infected cells were harvested 3 days postinfection. All baculovirus manipulations were performed as described previously (31). Capsids consisting of VP2 were purified from infected insect cells essentially as described previously (4) except that a 28% (wt/wt) CsCl gradient was used as the last purification step. This material was centrifuged to equilibrium at 100,000 × *g* for 24 h at 5°C. The banded material was dialyzed against three changes of phosphate-buffered saline (PBS), and the protein concentration was determined

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by the Bradford method (1a). The purified capsids were analyzed in a Philips CM12 electron microscope after negative staining with 0.05 M uranyl acetate (pH 3.6). The purity was checked in sodium dodecyl sulfate (SDS)-polyacrylamide gels.

Production of MAbs. The production of PAR1 (36), PAR3 (36), and BE11 (24) has been described. The antigen used was B19 virus isolated from viremic serum, and antibodies were screened by an ELISA (PAR1 and PAR3) and a dot blot ELISA (BE11), using B19 virus as the antigen. For these three MAbs, ascites fluid was obtained; in the case of PAR1, this material had been protein A-column purified.

MAbs D1 to D15 were produced by injecting two 8-week-old BALB/c mice intraperitoneally with 10 μg of purified VP2 capsids in Freund's incomplete adjuvant on days 0 and 14. The mice were boosted again on days 28 and 42 with 20 μg of VP2 in Freund's incomplete adjuvant. The spleens were removed, and two separate fusions were made with SP2/0-Ag8 myeloma cells, using polyethylene glycol 4000. The hybridoma cells were examined for the production of anti-B19 virus MAbs by an ELISA, using purified VP2 capsids coated on the microtiter plates. Positive clones were recloned twice, and hybridoma supernatants were used for testing. MAbs 3A2 and 3B2 were the gift of Jaap Middeldorp (Organon) and were produced by using a bacterial B19 virus–β-galactosidase fusion protein which contains a truncated VP1 protein up to nucleotide residue 4619 (28). Protein A-column-purified ascites fluid was obtained for testing.

Construction of pGEX expression plasmids. Plasmid DNA manipulations were performed as described previously (18). Plasmid transformations were carried out in *Escherichia coli* pC2495 (a derivative of JM101 obtained from Phabagen, Utrecht, The Netherlands). Restriction enzymes, the Klenow large fragment of DNA polymerase, T4 DNA ligase, and deoxynucleoside triphosphates were obtained from Pharmacia. Calf intestinal alkaline phosphatase was obtained from Boehringer Mannheim. The pGEX plasmid expression vectors pGEX-2T and pGEX-3X (described in reference 29) were obtained from Pharmacia. They direct the synthesis of foreign polypeptides in *E. coli* as fusions with the C terminus of a 26-kDa glutathione *S*-transferase (GST) from the helminth *Schistosoma japonicum*, under the control of an isopropylthiogalactopyranoside (IPTG)-inducible *tac* promoter. A polylinker that replaces the termination codon of Sj26 contains unique *Bam*HI, *Sma*I, and *Eco*RI recognition sites followed by TGA translation termination codons in all three reading frames. pGEX-2T contains the cleavage recognition sequence of the protease thrombin, and pGEX-3X contains that of factor X, which increases the molecular weight of GST to 275,000. These sites can be used to cleave the expressed polypeptide from GST after purification. pGEX-3X has a shift in the reading frame of the polylinker of +1 compared with pGEX-2T, and both vectors were used to obtain all B19 virus VP2 fusion proteins in frame with GST.

Seven pGEX clones expressing overlapping polypeptides of B19 virus VP1, designated VPG1 to VPG7, were generated. The source of B19 virus DNA, the isolation of which has been described previously (3), was a 2.49-kb *Hin*dIII-*Sca*I fragment between nucleotides 2430 and 4920 containing the complete coding region for VP1 (all nucleotide numberings are as described in reference 27) which had been ligated in the *Hin*dIII-*Sma*I sites of pUC19. For the expression of fusion proteins VPG1, VPG2, VPG4, VPG6, and VPG7, the respective fragments were made blunt (when necessary) and ligated in the *Sma*I site of pGEX-3X. The fragments encoding the fusion proteins were as follows: VPG1, a 510-bp *Sau*3A-*Pvu*II fragment between nucleotides 2570 and 3050; VPG2, an 820-bp *Hin*dII fragment between nucleotides 2880 and 3700; VPG4, a 510-bp *Nar*I-*Asp* 718 fragment between nucleotides 3570 and 4080; VPG6, a 580-bp *Sau*3A-*Eco*RI fragment between nucleotides 4340 and 4920; and VPG7, a 280-bp *Hin*fI-*Eco*RI fragment between nucleotides 4640 and 4920 (for VPG6 and VPG7, the *Eco*RI site originates from the pUC19 polylinker, as the *Sma*I site has been destroyed). For the expression of VPG3, a 790-bp *Hae*III fragment between nucleotides 3210 and 4000 was isolated and ligated in the blunt-ended *Eco*RI site of pGEX-3X. For the expression of VPG5, a 500-bp *Bam*HI-*Pvu*II fragment between nucleotides 3900 and 4400 was ligated in the *Bam*HI-*Sma*I sites of pGEX-2T. After transformation, clones containing the correct inserts were selected by restriction enzyme analysis.

SDS-PAGE analysis of proteins. *S. frugiperda* cells were infected with the recombinant baculovirus expressing both VP1 and VP2 as described above, and total proteins in 2×10^4 cells were analyzed. For the pGEX fusion proteins, SDS-polyacrylamide gel electrophoresis (PAGE) analysis was used to screen for the correct orientation of the inserted VP1 DNA fragment. Fragments in the opposite orientation produce only GST, while those in the correct orientation produce fusion proteins of various sizes which are larger than the GST control. Individual colonies from the various clones were grown overnight at 37°C in Luria broth containing 25 μg of ampicillin per ml. These fresh cultures were diluted 1:10 in Luria broth containing ampicillin and grown for 1 h at 37°C before addition of IPTG to 0.1 mM. Incubation was continued for 3 h, and the bacteria from 1 ml of culture were pelleted and taken up in 40 μl of SDS sample buffer (consisting of 2.5% SDS, 5% glycerol, 2.5% 2-mercaptoethanol, 62.5 mM Tris-Cl, and 0.05% bromophenol blue [pH 6.8]). Ten-microliter amounts were analyzed in SDS–10% polyacrylamide gels (17) stained with fast green (Fig. 1B).

ELISA. Twenty-nanogram amounts of VP2 and VP1/2 capsids were coated in PBS (pH 7.4) overnight at 4°C onto microtiter plates (Polysorp; Nunc). Plates were washed with PBS containing 0.05% Tween 20 and incubated for 1 h at 37°C with the MAbs diluted 1:10,000 (PAR1, PAR3, and BE11), 1:1,000 (D1 to D9 and 3A2), or 1:100 (D10 to D15) in PBS (pH 7.4) containing 2% fetal calf serum, 0.05% Tween 20, and 0.01% methiolate (PFTM). The plates were washed and incubated for 30 min at 37°C with a 1:4,000 dilution in PFTM of peroxidase-labelled rabbit anti-mouse immunoglobulin (Ig) G (DAKO A/S, Glostrup, Denmark). After washing, the substrate *o*-phenylenediamine (Abbott) was added, and the mixture was incubated at room temperature for 30 min. The reaction was stopped with 4 N H_2SO_4, and the A_{492} was read.

RIA. The radioimmunoassay (RIA) was performed in duplicate as an IgM capture assay as described previously (8). After every step, plates were washed with PBS containing 0.05% Tween 20 (PBST), and the sera, B19 virus, and MAbs were diluted in PBST containing 10% fetal calf serum. Microtiter plates were coated overnight at 4°C with goat anti-human μ-chain serum (TAGO) in 0.05 M sodium bicarbonate buffer (pH 9.6). The plates were washed, 100 U of B19 virus-specific IgM (as determined in the RIA) was added, and the plates were incubated for 3 h at 37°C. The plates were again washed, 0.2 ml of B19 virus antigen (isolated from the plasma of a viremic donor) or control antigen (prepared from the plasma of a nonviremic donor)

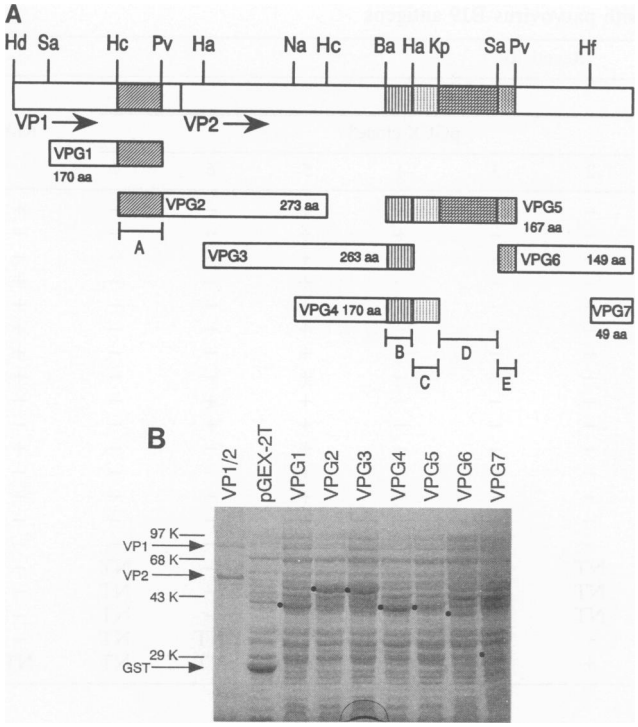

FIG. 1. (A) Positions of the pGEX fusion proteins VPG1 to VPG7 with respect to VP1 and regions to which MAbs bind; (B) SDS-polyacrylamide gel analysis of fusion proteins and baculovirus-produced VP1 and VP2. In panel A, the region to which MAbs 3A2 and 3B2 bind is designated A, the region to which MAbs D11 and D12 bind is designated B, the region to which MAb PAR3 binds is designated C, the region to which MAbs PAR1, BE11, D1 to D5, and D8 to D10 bind is designated D, and the region to which MAbs D6 and D7 bind is designated E. Restriction sites: Ba, *Bam*HI; Ha, *Hae*III; Hc, *Hin*cII; Hd, *Hin*dIII; Hf, *Hin*fI; Kp, *Kpn*I; Na, *Nar*I; Pv, *Pvu*II; Sa, *Sau*3A. aa, amino acids. In panel B, the positions of GST, VP1, and VP2 are indicated, and circles indicate positions of the fusion proteins.

was added to each well, and the plates were incubated for 3 h at 37°C. After washing, 0.2 ml of the MAb to be tested at 1:1,000 and 1:10,000 dilutions was added, and the plates were incubated overnight at 4°C. The plates were washed, 0.2 ml of ^{125}I-labelled sheep anti-mouse Ig (Amersham) was added, the plates were incubated for 2 h at 37°C and washed, and bound radioactivity was measured.

Western immunoblot analysis. Total proteins in insect cells expressing both VP1 and VP2 and in bacteria expressing the pGEX-VP1 fusion proteins were run in SDS-polyacrylamide gels as described above. The proteins were transferred onto a Zeta-probe nylon membrane (Bio-Rad), and the reactions were performed as described previously (3). The conjugate was alkaline phosphatase-labelled goat anti-mouse Ig (Promega). The blots were probed with the MAbs at the following dilutions: PAR1, PAR3, BE11, 3A2, and 3B2, 1:1,000; D1 to D9, 1:100; and D10 to D15, 1:50.

PEPSCAN. Overlapping nonapeptides covering amino acids 292 to 426 of the VP2 protein were synthesized and tested as described previously (11, 12). The first nonapeptide consisted of residues 292 to 300, the second consisted of residues 293 to 301, and so on. All peptides were tested against the MAbs in an ELISA. The ELISAs were repeated for those MAbs that gave a positive signal. Optical density values were read at 450 nm.

RESULTS

Reactivities of MAbs with native and recombinant parvovirus B19 particles. The reactivities of a panel of 19 MAbs were tested with baculovirus-produced B19 virus capsid proteins and native virus, using an ELISA and an RIA (Table 1). MAbs PAR1, PAR3, and BE11, which were raised against native B19, and D1 to D15, which were raised against baculovirus-produced VP2 capsids, reacted with the VP2 and VP1/2 capsids in the ELISA. These MAbs therefore recognize VP2-specific determinants, which is expected for D1 to D15 since these MAbs were raised against capsids consisting of VP2 alone. MAbs 3A2 and 3B2, which were raised against a bacterial β-galactosidase–VP1 fusion protein, reacted with the VP1/2 capsids in the ELISA and not the VP2 capsids and are therefore VP1 specific, as also shown by Western blot analysis (3) (Fig. 2).

The reactivities of the MAbs with native B19 virus in an RIA were tested to determine whether the determinants recognized by the MAbs made against the VP2 capsids are also found on the native virus. Of the 15 VP2-specific MAbs, D1 to D5 and D8 gave a positive reaction (D1 to D4 at a 1:10,000 dilution and D5 and D8 at a 1:1,000 dilution). Of the three MAbs that were raised against native virus, only PAR1 (at a 1:1,000 dilution) reacted with the B19 virus isolate used in the RIA.

Antigenicity of bacterial fusion proteins. The binding domains of the MAbs were localized by testing their reactivities with pGEX-GST fusion proteins in Western blots. Seven overlapping fusion proteins (VPG1 to VPG7) spanning the complete VP1 protein (Fig. 1A) were expressed in bacteria by cloning fragments of VP1 DNA at existing restriction sites in the pGEX expression vectors pGEX-2T and pGEX-3X. The fusion proteins produced are shown in Fig. 1B. It can be seen that the different proteins are produced in different amounts. The VPG7 fusion protein cannot be distinguished, and its presence and size were confirmed by affinity chromatography purification on immobilized glutathione (29). VPG1 and VPG5 could also be purified in this way, suggesting that these three polypeptides represent soluble areas of the VP1 protein (data not shown). Each MAb was tested in Western blots with baculovirus-expressed VP1 and VP2, pGEX-2T (as a control for reactivity with GST), and the fusion proteins (Table 1). None of the MAbs showed any reactivity with GST or any other bacterial proteins, confirming the specificity of the Western blots. 3A2 and 3B2 reacted only with VP1 in a Western blot and were therefore tested only with VPG1 and VPG2, since these fusion proteins cover the N-terminal unique part of VP1. They reacted with both VPG1 and VPG2, which localized the region to which they bind to a stretch of 57 amino acids (region A in Fig. 1A). The reactivity of 3A2 is shown in Fig. 2A.

The rest of the MAbs (except D13 to D15) reacted with both VP1 and VP2 in the Western blot, confirming the results from the ELISA showing that they recognize determinants on VP2. D13 to D15 reacted with the capsids in the ELISA and probably recognize conformational epitopes. Of the MAbs that reacted with VP1 and VP2 in the Western blot, D11 and D12 reacted with VPG3 to VPG5, which localized their binding site to a stretch of 33 amino acids (region B in Fig. 1A). The reactivity of D12 is shown in Fig. 2B. PAR3 reacted with VPG4 and VPG5, which localized its binding site to a stretch of 28 amino acids (Fig. 1A, region C). Its reactivity is shown in Fig. 2C. Ten MAbs (PAR1, BE11, D1 to D5, and D8 to D10) reacted with VPG5 alone in the

TABLE 1. Reactivities of MAbs with parvovirus B19 antigens

MAb	Binding domain[a]	Reactivity									
		ELISA		Western blot								RIA
					pGEX clone[b]							
		VP2	VP1/2	VP1/2	1	2	3	4	5	6	7	
PAR1	D	2.861	2.329	+	−	−	−	−	+	−	−	+
PAR3	C	2.811	2.363	+	−	−	−	+	+	−	−	−
BE11	D	>2.990	2.827	+	NT[c]	−	−	−	+	−	−	−
D1	D	1.862	1.602	+	NT	−	−	−	+	−	−	+
D2	D	1.386	1.363	+	NT	−	−	−	+	−	−	+
D3	D	1.905	1.636	+	NT	−	−	−	+	−	−	+
D4	D	2.537	2.069	+	NT	−	−	−	+	−	−	+
D5	D	0.680	0.489	+	NT	−	−	−	+	−	−	+
D6	E	0.436	0.252	+	−	−	−	−	+	+	−	−
D7	E	0.782	0.398	+	−	−	−	−	+	+	−	−
D8	D	0.624	0.421	+	NT	−	−	−	+	−	−	+
D9	D	0.359	0.216	+	NT	−	−	−	+	−	−	−
D10	D	1.005	0.617	+	−	−	−	−	+	−	−	−
D11	B	>2.990	>2.990	+	−	−	+	+	+	−	−	−
D12	B	2.740	2.121	+	−	−	+	+	+	−	−	−
D13		0.932	0.800	−	NT	NT	NT	−	−	−	NT	−
D14		0.716	0.471	−	NT	NT	NT	−	−	−	NT	−
D15		1.326	1.101	−	NT	NT	NT	−	−	−	NT	−
3A2[d]	A	0.046	0.475	+	+	+	NT	NT	NT	NT	NT	−
3B2[d]	A	NT	NT	+	+	+	NT	NT	NT	NT	NT	NT

[a] As shown in Fig. 1A.
[b] 1 to 7 represent VPG1 to VPG7.
[c] NT, not tested.
[d] Did not react with VP2 in the Western blot.

Western blot, which localized their binding sites to a stretch of 84 amino acids shown as region D in Fig. 1A. The reactivity of PAR1 is shown in Fig. 2D. Lastly, D6 and D7 reacted with VPG5 and VPG6, which cover a 22-amino-acid stretch (region E in Fig. 1A). The reactivity of D6 is shown in Fig. 2E. Since this MAb reacted strongly with VPG5 and weakly with VPG6, just before development of the color reaction, the filter was cut in strips to separate the lanes containing VPG5 and VPG6 from the rest of the samples. In repeat experiments, in which the filters were intact and thus developed to the same extent, the reactivity of D6 remained specific for the two fusion proteins. The same was true for D7. The extra bands seen in the Western blots are due to breakdown products.

PEPSCAN analysis. To precisely map the binding sites of the MAbs that bind to regions C, D, and E, a PEPSCAN was performed. Overlapping nonapeptides covering amino acids 292 to 426 of the VP2 protein were synthesized and tested as described above. Three MAbs reacted positively (Fig. 3). The binding site of D2 covered six peptides and was localized to a stretch of 14 amino acids between residues 321 and 334 of VP2. D10 bound to six peptides covering the 14 amino acids between 347 and 360, and PAR1 bound to eight peptides covering amino acids 354 to 369, a stretch of 16 amino acids. 3A2, which served as a negative control, did not react with any of the peptides analyzed in the PEPSCAN. None of the remaining MAbs tested (PAR3, BE11, D1, and D3 to D9) gave a signal in the PEPSCAN ELISA, which suggests that their binding sites are longer than 9 amino acids (BE11 has been previously mapped to a peptide of 17 amino acids [24]) or that some degree of conformation is involved in the recognition site.

DISCUSSION

Binding sites of the MAbs and localization of an immunodominant domain on the VP2 capsids. A panel of 20 MAbs was used to map epitopes on recombinant parvovirus B19 virus VP2 particles. The VP2 specificities of the 15 MAbs made against the VP2 capsids (D1 to D15) were confirmed by testing their reactivities in an ELISA with purified baculovirus-produced VP2 and VP1/2 capsids. The three MAbs made against the native B19 virus (PAR1, PAR3, and BE11) were also shown to recognize epitopes on VP2 in this way. The two MAbs (3A2 and 3B2) that did not react with VP2 in the ELISA were VP1 specific, as shown by Western blot analysis. Of the 15 MAbs raised against recombinant VP2 particles, 12 had sequential binding domains concentrated within a 167-amino-acid stretch of the VP2 protein between amino acids 259 and 426, as shown by their reactivities with bacterial fusion proteins expressing overlapping fragments of VP1. This finding suggests that this is an important immunogenic region of the recombinant VP2 capsids. The three MAbs (D13 to D15) that reacted with the VP2 and VP1/2 capsids in the ELISA but not with the same antigens in a Western blot probably recognize conformational epitopes. Within this 167-amino-acid stretch, the binding sites of four MAbs (D11, D12, D6, and D7) were mapped to discrete peptide fragments ranging from 22 to 33 amino acids (domains B and E; Fig. 1A). Domain D, an 84-amino-acid stretch within the immunogenic region, was the binding domain for eight of the MAbs (D1 to D5 and D8 to D10), which suggests it is the most important antigenic domain within this 167-amino-acid region. The three MAbs raised against native B19 virus also recognize epitopes within the 167-amino-acid region. Three MAbs, D2, D10, and PAR1,

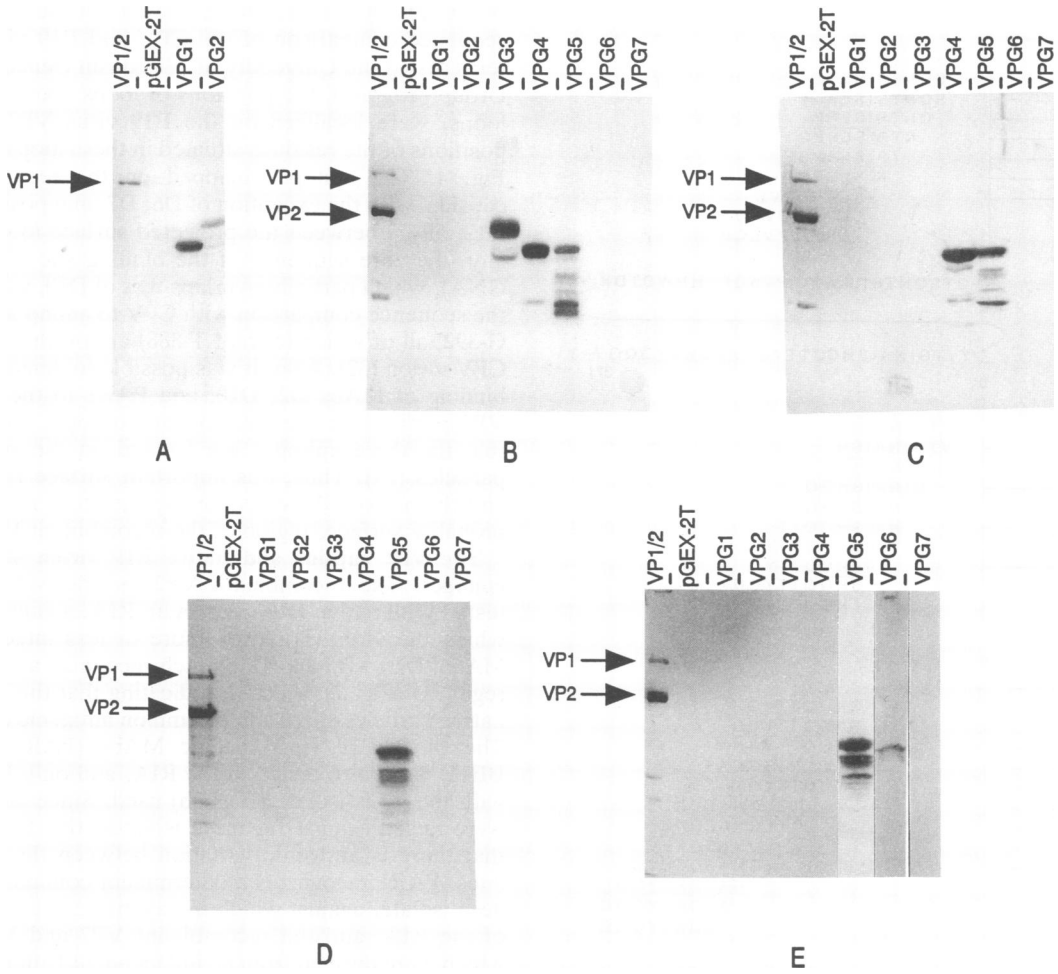

FIG. 2. Western blots showing reactivities of the MAbs with VP1 and VP2 produced in insect cells and the pGEX fusion proteins. (A) Reactivity of 3A2 at a 1:1,000 dilution; (B) reactivity of D12 at a 1:50 dilution; (C) reactivity of PAR3 at a 1:1,000 dilution; (D) reactivity of PAR1 at a 1:1,000 dilution; (E) reactivity of D6 at a 1:50 dilution (the binding patterns shown correspond to binding regions A to E in Fig. 1A).

could be fine-mapped within domain D by using a PEPSCAN (Fig. 3 and 4). An analysis of the peptides recognized by D2 and PAR1 made it possible to identify a core sequence of six amino acids (ISLRPG for D2 and TTYGNA for PAR1) that may be the essential residues for binding (Fig. 3, boxed residues). The binding site of D10 is a little more complex. The core sequence ISHG (residues boxed by a continuous line in Fig. 3) is present in all peptides which give a signal in the PEPSCAN ELISA. In addition, two residues at the extreme ends of the first and last positive peptides (TG and YG, respectively) are responsible for a significant increase in signal (residues boxed by dotted lines in Fig. 3). The presence of either of these end residues may result in the peptide adopting a conformation that enhances binding of the antibody.

The immunodominant domain on the VP2 capsids correlates with an important surface region on the native B19 virion. Evidence that the 167-amino-acid region on the recombinant VP2 capsids is also an important antigenic domain of the native B19 virion comes from a previous study in which peptides from hydrophilic regions of the VP2 protein were synthesized. These peptides were used to purify peptide-specific antibodies from human sera containing B19 virus-specific IgG, and the antibodies were subsequently tested for the ability to neutralize and immunoprecipitate native virus (25). Five of these epitopes map to the antigenic region defined on the recombinant VP2 capsids. Four (S253, S309, S325, which is also the recognition site for BE11, and S359) were recognized by human antibodies that can both neutralize and immunoprecipitate native virus, and one (S288) was recognized by antibodies that can immunoprecipitate native virus. This region is therefore assumed to be on the surface of the native virion. The positions of these epitopes in relation to the epitopes mapped on the recombinant VP2 capsids are shown in Fig. 4; there is a high degree of overlap except for D6 and D7. The pGEX fusion protein VPG5, encompassing binding domains B to E of the VP2 capsids, was shown to be soluble, and therefore hydrophilic, by its purification by affinity chromatography on immobilized glutathione (29). Further, MAb PAR1 can immunoprecipitate native virions and MAb BE11 can neutralize virus infectivity, suggesting that they recognize surface epitopes. They both also recognize epitopes located in domain D of the antigenic region on the recombinant VP2 capsids. These lines of evidence indicate that this antigenic region is on the surface of the recombinant VP2 capsids.

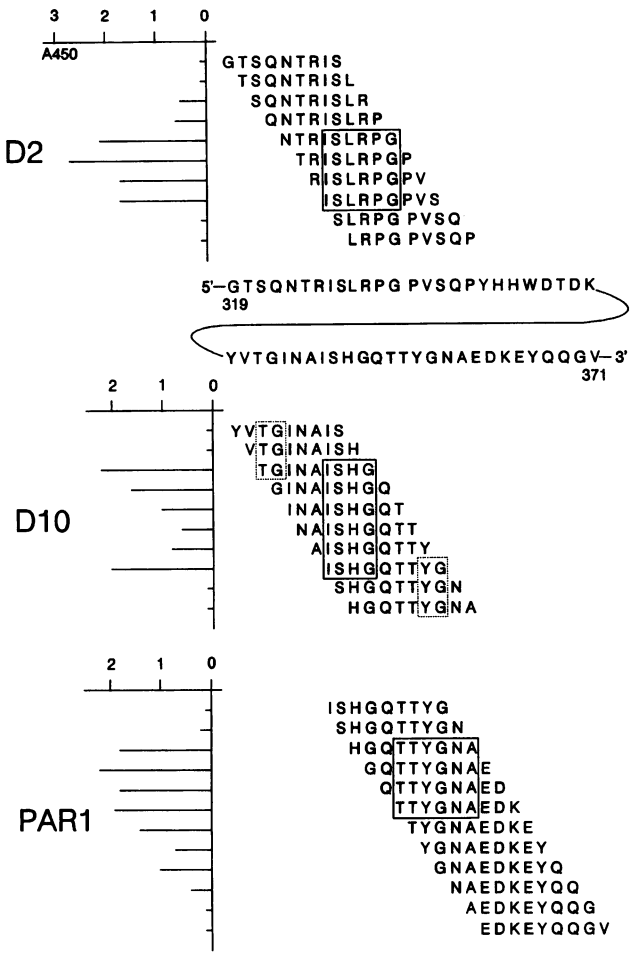

FIG. 3. PEPSCAN of amino acids 319 to 371 of VP2 reacted with MAbs D2, D10, and PAR1. The peptides and amino acids (boxes with solid and dotted lines) to which the three MAbs bind are shown. The vertical axis shows A_{450} values. D2 was tested at a 1:50 dilution, D10 was tested at a 1:2 dilution, and PAR1 was tested at a 1:1,500 dilution.

The immunodominant domain on the VP2 capsids correlates with the surface spike of CPV. In canine parvovirus (CPV), the only parvovirus for which the three-dimensional structure is known, a major part of the virion surface is made up of the threefold spike which consists of four loops (32). By sequence comparison of the CPV and B19 virus VP2 proteins (using the University of Wisconsin Genetics Computer Group program), the positions of loops 3 and 4, the largest loops, were localized on the B19 virus VP2 protein; the positions of the residues situated in these loops are shown in Fig. 4. The epitopes mapped on the recombinant VP2 capsids, with the exception of D6, D7, and possibly D11 and D12, all lie between the projected surface loops. Additionally, the core sequence of the binding site of PAR1 (TTY GNA), which partially overlaps that of D10, was matched in the sequence comparison with CPV to amino acids K-387 to G-392, all of which (except T-388) are on the surface of the CPV virion (32). Also, it was possible to demonstrate direct binding of MAbs D2, D10, and PAR1 to the recombinant VP2 particles in the electron microscope, which confirms that the three epitopes are located on the surface of the particles (34). Thus, this important surface region of CPV and the native B19 virus corresponds to a major antigenic, probably surface, region of the recombinant VP2 capsids.

The VP2 capsids and native B19 virion share common epitopes. The recombinant VP2-specific MAbs were also tested with native B19 virus in an RIA, a capture system in which the virus should be more or less intact. Six of the MAbs, D1 to D5 and D8, which recognize a determinant in region D (Fig. 2), reacted, indicating that the synthetic and native particles share some common antigenic determinants. The three B19 virus-specific MAbs (PAR1, PAR3, and BE11) were also tested in the RIA, and only PAR1 reacted with the virus isolate (English) used. Since all three MAbs were raised against Japanese isolates, this finding suggests that there is antigenic variation between the different isolates. PAR1 recognizes a determinant common to the Japanese isolate, against which it was made, the English isolate of the RIA, and the recombinant VP2 and VP1/2 capsids which were derived from an independent Dutch virus isolate (3). PAR3 and BE11 both reacted with the VP2 and VP1/2 capsids in the ELISA; therefore, these particular determinants are common to the Japanese strains and the recombinant capsids derived from the Dutch virus isolate. Similarly, the VP2-specific MAbs (D6, D7, D9, and D10) that did not react in the RIA may well react with other isolates of B19 virus. Such variation between isolates has been shown only at the DNA level by restriction enzyme analysis (19, 20), but extensive sequence analysis of different isolates to detect amino acid substitutions has not been performed.

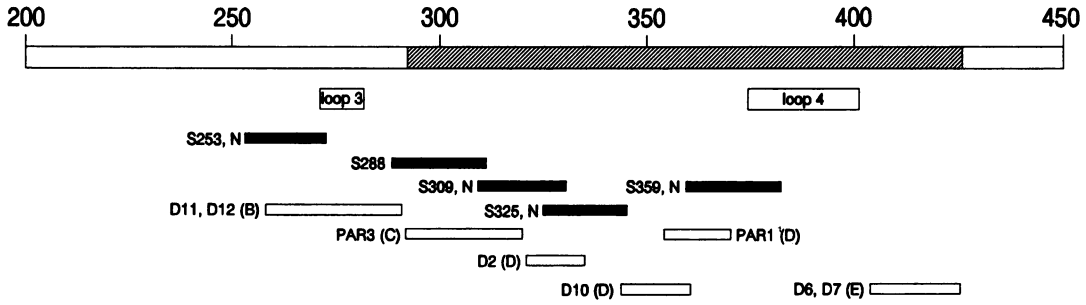

FIG. 4. Positions of the mapped determinants in relation to previously mapped B19 virus epitopes and the putative surface loops. The numbers 200 to 450 refer to VP2 amino acid residues; the shaded region is the area of the PEPSCAN; the two boxes show loops 3 and 4 extrapolated from the structure of CPV and constituting part of the spike region; thin black boxes represent epitopes mapped previously (25) and have the same nomenclature, N shows neutralizing epitopes (S325 is the determinant also recognized by BE11); thin white boxes represent epitopes mapped on the recombinant VP2 capsids, with names of the MAbs and the regions to which they bind as shown in Fig. 1A.

The fact that the VP2 (and VP1/2) capsids and native B19 virus have common antigenic domains and determinants, and possibly surface topology, is important if these capsids are to be used as a vaccine against B19 virus. In view of this consideration, the ability of the VP2 and VP1/2 capsids to induce neutralizing antibodies may need to be determined. Such a vaccine could be applied to specific high-risk patient groups, such as sufferers of sickle cell anemia and other types of hemolytic anemia in which a B19 virus infection can cause an aplastic crisis, and to prevent fetal death. Another possible use of the VP2 capsids is as a vaccine carrier for the presentation of foreign epitopes to the immune system, as has been described for hepatitis B virus core (7, 10, 30), and surface (22) antigens and yeast Ty virus-like particles (13). The recombinant VP2 capsids are immunogenic in both mice (this report) and rabbits, in which high-titered polyclonal sera have been produced (data not shown), and can be easily produced and purified in large amounts, approximately 10 mg/10^9 cells. As for the positions of insertion of the foreign sequences, studies with hepatitis B virus core antigen have shown that insertion of epitopes in an immunodominant region of the particle, which is also surface exposed, renders these epitopes more immunogenic than the same epitopes fused to the N terminus (2, 26). For the VP2 capsids, for which the three-dimensional structure is not known, the well-defined sites recognized by MAbs D2, D10, and PAR1 may represent such immunoexposed determinants that would result in a high immunogenicity of inserted sequences.

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