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Prokaryotic Expression of a VP1 Polypeptide Antigen for Diagnosis by a Human Parvovirus B19 Antibody Enzyme Immunoassay

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To produce parvovirus B19 antigen for diagnostic purposes, partially overlapping segments covering the genes encoding the viral structural proteins VP1 and VP2 were cloned into expression vectors. The constructs were induced in *Escherichia coli*, resulting in the expression of β-galactosidase fusion proteins. In immunoblotting experiments with sera from patients with erythema infectiosum, immunoglobulin G (IgG) and IgM antibodies bound to a single polypeptide of 235 amino acids at the N terminus of VP1. The DNA fragment encoding this polypeptide was amplified by the polymerase chain reaction and cloned into an expression vector. The viral capsid antigen expressed in *E. coli* was purified by preparative agarose gel electrophoresis and used in IgG and IgM solid-phase enzyme immunoassays. Comparison with reference γ- and μ-capture radioimmunoassays using whole virus antigen showed that these antibody tests are suitable for the serodiagnosis of human infections caused by parvovirus B19.

Human parvovirus B19 is a member of the genus *Parvovirus* (antonomous parvoviruses) of the family *Parvoviridae* (38). The other two genera of this family are *Dependovirus* (or adeno-associated virus) and *Densovirus* (infects insect cells only). With a diameter of about 20 nm, parvoviruses are among the smallest DNA viruses that infect animal cells. Their genome is a linear single-stranded DNA molecule of about 5.5 kb which may be of either polarity (11). The nonenveloped capsid of B19 consists of two major proteins, VP1 (4%) and VP2 (96%), the latter being completely included in the former (24, 26).

Parvovirus B19 was discovered by Cossart et al. in 1975 while screening blood donor sera for hepatitis B antigen (13). In 1981, this virus was established as an important cause of aplastic crisis in children with sickle cell anemia (27, 36). Other diseases caused by B19 include erythema infectiosum (fifth disease), a common childhood exanthem (4, 28), and arthritis, which occurs preferentially in adults (30, 44). During pregnancy, intrauterine infection by B19 may result in fetal anemia, hydrops fetalis, spontaneous abortion, or intrauterine death (1, 9, 15, 18, 43). In immunodeficient subjects, B19 may establish chronic bone marrow infections manifesting as persistent reticulocytopenic anemia (19).

Currently, sera from blood donors provide the main source of antigen for diagnostic purposes, since patients are no longer viremic during erythema (2, 3, 12). Attempts to grow human parvovirus B19 in established cell lines have been unsuccessful because of the specific tropism of the virus for erythroid progenitor cells. Propagation of the virus in primary cultures of such cells from bone marrow or from fetal liver is possible (8, 25, 40, 46), but, because of the limited availability of the cells and the low yield of virus, that method does not appear suitable for large-scale antigen production. Recombinant parvoviral capsids have, however, been expressed in transfected Chinese hamster ovary (CHO) cells (17) and in baculovirus-infected insect cells (7).

The goal of this study was to obtain antigen in order to establish diagnostic antibody tests for human parvovirus B19 infections.

MATERIALS AND METHODS

Viral DNA and cloning strategy. Parvoviral DNA, originating from viremic human serum, was purified by ultracentrifugation and proteinase K treatment and then by *Aat*II and *Nde*I digestion and inserted into a cloning plasmid together with a synthetic 110-nucleotide (nt) sequence constituting the missing last portion of the 3′ end, as described previously (29). Such a plasmid, pDEV119, containing both genes for the capsid proteins VP1 and VP2 (nt 2430 to 4790, according to the sequence determined by Shade et al. [37]), was obtained from F. B. Rayment (29). We first excised several partially overlapping fragments from this plasmid, covering all of VP2 and VP1 from nt 2581 (Fig. 1, *Ssp*I site) corresponding to amino acids 47 to 781, cloned and expressed them in the *lac*Z fusion expression vectors pEX1, −2, and −3 (41) and pDEV107 (obtained from F. B. Rayment), and studied the expression and immunogenicity of the products by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting, respectively.

In order to excise the extreme 5′ end of VP1 (nt 2444 to 3124) in a translatable form, the *Hin*dIII site could not be used because of a TAG stop codon upstream of the initiation site (ATG) of VP1 (Fig. 1). To overcome this stop codon, new restriction sites were created by using the polymerase chain reaction (PCR) (23, 32). The new restriction sites (*Bam*HI and *Sal*I) were synthesized as part of the PCR primers, *Bam*HI in the 5′ primer (nt 2444 to 2467) and *Sal*I in the 3′ primer (nt 3127 to 3149), and were then integrated into the VP1 gene during PCR amplification. The DNA was amplified in 25 cycles, each consisting of denaturation for 1.5 min at 93°C, hybridization of the template and primers for 1 min at 40°C, and polymerization of the new DNA strands for 5 min at 70°C. In the reaction, 2.5 U of *Taq* polymerase (Cetus, Emeryville, Calif.); 200 μM each dATP, dCTP, dGTP, and dTTP; 1 μM each primer; and 0.1 to 1 ng of template (pDEV119, containing the full-length viral VP1)

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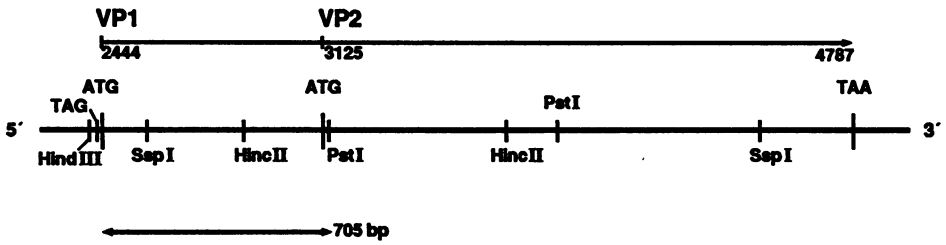

FIG. 1. Genes VP1 and VP2 of B19. TAG is a stop codon, ATG is the initiation codon, and TAA is the termination codon. The 705-bp gene fragment cloned in pEX706 is shown.

were incubated in 10 mM Tris-HCl buffer, pH 8.3, containing 50 mM KCl, 1.5 mM $MgCl_2$, and 0.01% gelatin. The 720-bp amplification product was purified by phenol and ether extraction and ethanol precipitation and then digested with *Bam*HI and *Sal*I, resulting in a fragment of 714 bp.

The digested fragment was purified by preparative agarose gel electrophoresis and then recovered by heating in phenol, ether extraction, and ethanol precipitation. It was then ligated to *Bam*HI-*Sal*I-digested pEX1, pDEV107, and pJLA502 (GBF/Medac, Hamburg, Germany) expression vectors to obtain pEX706, pDEV706, and pJLA706, respectively. These constructs contain 705 bp (nt 2444 to 3149) of parvovirus B19 and an extra nucleotide to give the correct reading frame. Unlike pDEV and pEX, pJLA is not a β-galactosidase fusion expression vector but produces native recombinant polypeptides by using λ P_R and P_L promoters (34).

Transformation of *Escherichia coli* and characterization of the DNA. *E. coli* cells were made competent by the $CaCl_2$ method described by Mandel and Higa (20), except that the cells were kept on ice for 1 to 16 h before transformation. The ligation mixture was diluted twofold in TE buffer (10 mM Tris-HCl, pH 8, containing 1 mM EDTA). Ice-cold DNA, 1 to 10 μl (5 to 50 ng), was mixed with 0.2 ml of competent cells, and the mixture was chilled for 30 min. The cells were incubated at 42°C (pEX at 34°C) for 1.5 min and rechilled on ice for 2 min. After one hour of incubation in Luria broth growth medium the cells were plated out on LB agar plates containing 50 μg of ampicillin (LB-ampicillin) per ml and grown for ≥16 h at 37°C (pDEV) or at 30°C (pEX and pJLA). Transformant colonies were inoculated into 5 ml of LB-ampicillin and were grown overnight before isolation of the DNA or induction for protein expression. Plasmids were isolated as described previously (5) and analyzed by restriction enzyme digestion and agarose gel electrophoresis.

The *Pst*I-digested plasmid pEX706 was blotted by the method of Southern and hybridized (21) overnight at 42°C with a ^{32}P-labeled *Hin*dIII-*Hin*cII VP1 fragment (nt 2430 to 2880) as a probe. This probe was excised form pDEV119, which contains the VP1 gene, and purified and separated from vector sequences by preparative agarose gel electrophoresis. The radiolabeling was carried out by nick translation (31) with Nick Translation kits (Amersham, Amersham, United Kingdom). The hybridized filters were washed in 1 × SSC (0.15 M NaCl, 0.015 M sodium citrate, pH 7)–0.1% SDS for 15 min at 22°C and three times for 30 min at 60°C; then they were washed for 30 min in 0.1× SSC–0.1% SDS at 60°C. Films were exposed for 5 to 16 h at −70°C.

The B19-VP1 DNA fragment of the pEX706 clone was sequenced directly from pEX706. The sequencing method applied was modified from the work of Sanger et al. (33) and follows the instructions of the Sequenase kit (USB Corporation). The double-stranded plasmid was first denatured in 0.5 M NaOH for 5 min and neutralized with 0.4 volumes of 3 M sodium acetate, pH 4.5, as modified from Chen and Seeburg (10) and Hattori and Sakaki (16).

Protein expression and immunoblotting. Overnight cultures of cells harboring the pEX or pJLA vectors were diluted 1:100 in LB-ampicillin and were grown for 4 h at 30°C. Subsequent induction was carried out by rapid shifting to 42°C for 90 min. Vector pDEV107 was induced by diluting the cells (1:5) in media containing 60 μg of isopropyl-β-D-thiogalactopyranoside per ml and then incubation for 5 to 8 h at 37°C.

The induced cells were centrifuged and resuspended in H_2O and then sample buffer (20% glycerol, 10% mercaptoethanol, 4% SDS, 0.02% bromphenol blue in 0.125 M Tris-HCl, pH 6.8). Heated aliquots were run in mini-SDS-polyacrylamide gels (7.5% acrylamide; Mighty Small 2; Hoefer Scientific Instruments, San Francisco, Calif.). The protein bands were visualized by Coomassie blue staining.

Alternatively, proteins in the SDS-PAGE gels were transferred (42) onto nitrocellulose filters (Schleicher & Schuell, Dassel, Germany) by using a TE Mighty Small Transfer Electroforesis unit (Hoefer). The filters were treated with TEN-Tween (5 mM EDTA, 150 mM NaCl, 0.05% Tween 20 in 50 mM Tris-HCl buffer, pH 7.4) for 30 min and then incubated for 1 h with human sera (1:100 in TEN-Tween) or with mouse monoclonal anti β-galactosidase antibody (1:10,000; Promega, Madison, Wis.). After washing with TEN-Tween, the filters were treated with peroxidase-conjugated, anti-human (or anti-mouse) immunoglobulin G (IgG) or IgM antibodies (1:100; DAKO, Glostrup, Denmark) for 1 h and then diaminobenzidine tetrahydrochloride (0.5 mg/ml) and H_2O_2 (0.4 mg/ml) in 50 mM Tris-HCl, pH 7.5. As a control antigen, we used β-galactosidase (116 kDa) expressed from pEX vectors lacking parvoviral DNA.

Antigen solubility. To study the solubility of the pEX706 translation product (designated βVP1), the bacteria were frozen and thawed three times and suspended in TNE buffer (100 mM NaCl and 1 mM EDTA in 10 mM Tris-HCl buffer, pH 8) containing 1 mg of lysozyme per ml for 20 min and in TNE with 0.05% deoxycholate for 10 min at 0°C. After centrifugation at 15,000 rpm for 10 min (Eppendorf), the pellet and supernatant fractions were studied by SDS-PAGE.

For further solubility studies, pellet fractions of the lysozyme-treated cells were carefully suspended in 8 M urea or 6 M guanidine hydrochloride (GuHCl) in TNE or in 2% SDS–5% β-mercaptoethanol (sample buffer). After centrifugation at 15,000 rpm for 10 min, the pellets and supernatants

were dialyzed and recentrifuged and the supernatants and pellets were studied by SDS-PAGE.

Purification of recombinant antigen. Induced cultures (100 ml) of *E. coli* harboring pEX706 (or pEX1 vector alone as a negative control) were centrifuged in a Sorvall centrifuge at 3,000 rpm for 10 min; the pellets were suspended in 1 ml of 50 mM Tris-HCl, pH 8.0, with 1 mM EDTA–50 mM NaCl, sonicated for 3 min, and centrifuged in an Eppendorf centrifuge at 15,000 rpm for 10 min. The pellets were resuspended in 1 ml of distilled water and sample buffer (1:2) and then boiled for 5 min. These mixtures were subjected to preparative vertical agarose gel electrophoresis (ProSieve agarose; FMC BioProducts, Rockland, ME) in separate gels for 2 h at 25 mA. Protein bands (140 kDa for the βVP1 recombinant fusion protein and 116 kDa for β-galactosidase) were visualized by precipitation with 1 M ice-cold potassium acetate or directly against light and were excised from the gel. The gel slices were melted in 1 ml of 50 mM Tris-HCl (pH 8.0)–1 mM EDTA in water bath at 80°C, carefully mixed, and placed on ice. The solidified gels were then frozen, thawed, and centrifuged in an Eppendorf centrifuge at 15,000 rpm for 15 min. The supernatants and pellets (dissolved in an equal volume) were studied by SDS-PAGE.

Enzyme immunoassay (EIA). The supernatant from the last purification step, containing the recombinant βVP1 antigen or β-galactosidase as a control was diluted (1:100) in phosphate-buffered saline (PBS), pH 7.5, and adsorbed onto plastic microwell plates (Labsystems, Helsinki, Finland) at 22°C overnight. The antigen-coated wells were washed 2 times for 5 min each with 8 M urea in PBS and then with PBS containing 0.05% Tween 20 three times for 10 min each. Sera in PBS-Tween 20, diluted 1:100 for IgG and 1:50 for IgM, were applied for 1 h at 37°C. After being washed, the wells were treated for 1 h with alkaline phosphatase-conjugated anti-human IgG or IgM (Orion Diagnostica, Espoo, Finland) and then with paranitrophenyl phosphate substrate (1 mg/ml in diethanolamine buffer containing 0.5 mM $MgCl_2$, pH 10) for 30 min at 22°C. The reaction was stopped with 100 μl of 1 M NaOH, and the A_{405} was read.

Patient sera. For antigenicity tests, human sera containing IgG and IgM antibodies against parvovirus B19 was obtained from Mary Anderson, University College Hospital, London, United Kingdom.

For evaluation of the EIA, sera were obtained from three groups of subjects. Group 1 consisted of children with a clinical suspicion of erythema infectiosum during outbreaks in Finnish schools and preschools and from the patients' close contacts. This group comprised 211 serum samples, of which 134 (2 × 67) were paired. Group 2 consisted of patients with arthritis and/or erythematous rashes, and it comprised 84 serum samples (including 14 pairs). This group represents typical material studied in a viral diagnostic laboratory. The additional clinical diagnoses of the patients were rubella, measles, mumps, herpes, urticaria, lymphadenopathy, cephalalgia, gastroenteritis, hepatitis, malaise, fever, eye infections, and respiratory symptoms. Nine other laboratory diagnoses had been obtained for group 2: cytomegalovirus, herpes simplex virus, influenza A virus, influenza B virus, parainfluenza virus, sindbis virus, and varicella-zoster virus. Group 3 consisted of nonsymptomatic members of the laboratory staff, with 87 single serum samples. The antibody statuses of all the 382 serum samples were reexamined by γ- and μ-capture radiommunoassays (Central Public Health Laboratory) as reference tests employing whole virus as antigen (12).

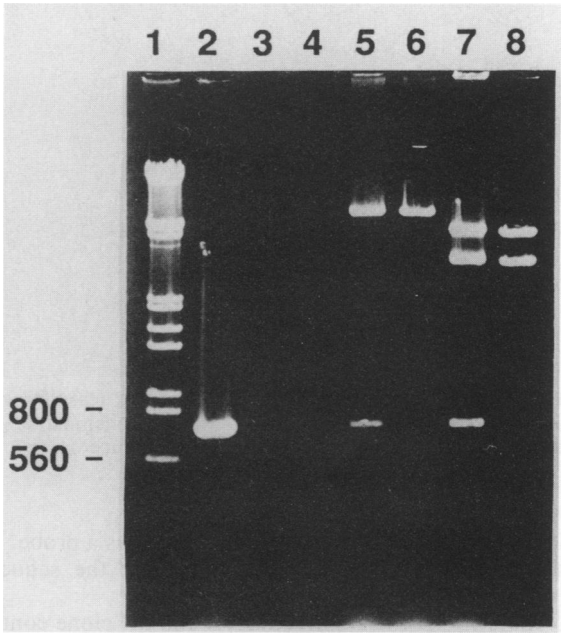

FIG. 2. DNA run is 1% agarose gel electrophoresis and stained with ethidium bromide. Lane 1, molecular weight (base-pair) markers; lanes 2 to 4, PCR results (lane 2 shows the PCR-amplified, 720-bp fragment; lanes 3 and 4 are the negative PCR controls; in lane 3, vector without B19 DNA was used as the template; in lane 4, the PCR reaction was performed without primers); lanes 5 and 6, positive and negative clones of pEX706, respectively; lanes 7 and 8, positive and negative clones of pDEV706, respectively (constructs in lanes 5 to 8 were digested with *Bam*HI and *Pst*I).

RESULTS

The entire VP2 gene and VP1 downstream from nt 2581 (Fig. 1, *Ssp*I site) of the VP1-VP2 gene sequence corresponding to the amino acids 47 to 781 was initially chosen for cloning and expression. In immunoblotting experiments, the expression products were labeled by mouse monoclonal anti-β-galactosidase antibodies. However, none of these proteins reacted with human anti-B19 IgG antibodies (data not shown).

The PCR-derived pEX706 and pDEV706 clones were analyzed by restriction enzyme digestion. As shown in Fig. 2, the amplified 720-bp DNA fragment, as well as the inserts of plasmids pEX706 and pDEV706, had the correct length. These positive clones were grown and induced to produce recombinant proteins. The nonfusion construct, pJLA706, did not express recombinant proteins (data not shown). However, both pEX706 and pDEV706 did express fusion polypeptides which migrated in SDS-PAGE at 140 kDa (Fig. 3a). This size corresponds to the theoretical molecular mass of the recombinant β-galactosidase (1,045 amino acids; ~116 kDa [41]) plus the VP1 region of 235 amino acids (~26 kDa).

In immunoblotting, these 140-kDa fusion proteins were heavily labeled by human anti-B19 IgG (Fig. 3b) and IgM (Fig. 4) antibodies, whereas the β-galactosidase control antigen was not (Fig. 3b, lanes 4 and 7, and Fig. 4, lane 1). Plasmid pEX706, rather than pDEV706, was chosen for antigen production because of a higher yield of expressed protein (Fig. 3a).

Characterization of pEX706 and βVP1. The amplified DNA fragment, as well as the purified cloned pEX706, was blotted by the method of Southern and hybridized with a

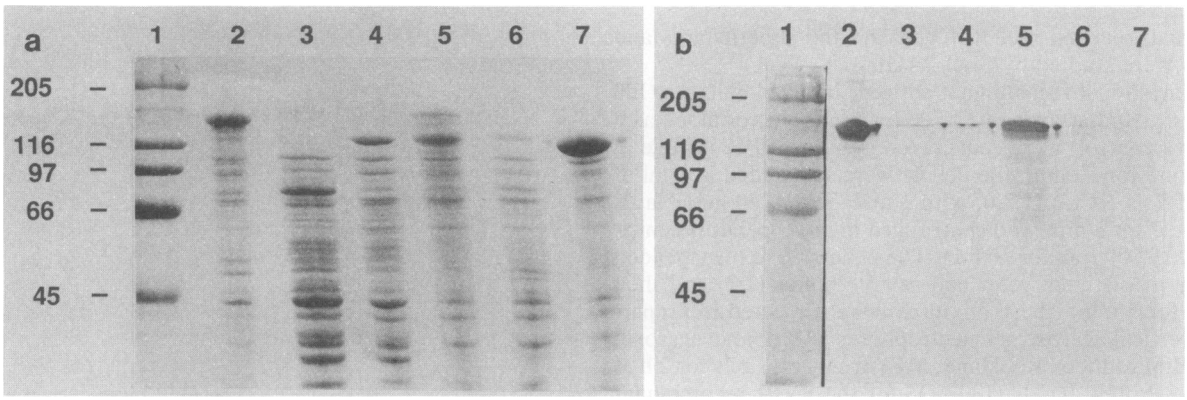

FIG. 3. Whole-bacterial lysates run in two identical SDS gels. (a) Coomassie blue staining; (b) immunoblotting with B19 IgG-positive human serum. Lanes: 1, molecular mass (kilodaltons) markers; 2, pEX706 induced; 3, pEX706 uninduced; 4, pEX1, induced; 5, pDEV706 induced; 6, pDEV706 uninduced; 7, pDEV107 induced.

^{32}P-labeled *Hin*dIII-*Hin*cII fragment of VP1 as a probe. The positive hybridization result suggested that the sequence was from parvovirus B19 (data not shown).

To confirm that the amplified DNA and the clone containing it had the correct B19 sequence and to see whether mutations had occurred during amplification, the B19 fragment was sequenced directly from the pEX706 plasmid. The sequence obtained was identical with the B19-Au sequence published by Shade et al. (37), except for the nts 2453 (A → G) and 2575 (T → C). The former replacement, which is the same as in the B19-Wi sequence (6), changes the fourth amino acid from lysine to glutamic acid. The latter change does not alter the amino acid sequence.

Finally, the solubility of the βVP1 expression product was investigated as described in Materials and Methods. In mild conditions (TNE-lysozyme and TNE-deoxycholate), the 140-kDa βVP1 fusion protein was retained in the insoluble pellet fraction. Urea solubilized it partially (50%), GuHCl solubilized it almost completely (90%), and SDS in reducing conditions solubilized it completely (data not shown). This enabled the purification of the recombinant antigen by preparative agarose gel electrophoresis. In order to excise the desired protein band from the gel, it was first visualized by potassium acetate. With this method, only ~60% of the βVP1 protein was recovered in the supernatant. However, this band was dense enough to be detected without staining.

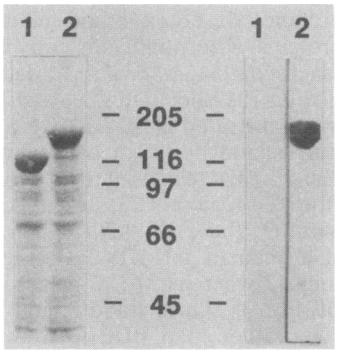

FIG. 4. SDS-PAGE and immunoblotting of whole-cell lysates. The filter on the left is strained with Ponceau, and the one on the right is immunoblotted with B19 IgM-positive patient serum. Lanes 1, induced vector pEX1, expressing β-galactosidase; 2, induced pEX706, expressing the βVP1 fusion protein.

From such untreated gels, a substantially greater yield (≥90%) of purified antigen was obtained.

EIA results. The purified βVP1 protein was employed as antigen in solid-phase EIA. Altogether, 382 serum samples were tested, and the results were compared with those obtained by the γ- and μ-capture RIAs of the Central Public Health Laboratory. The distribution of the βVP1 EIA results in all three subject groups is shown in Fig. 5. By using an A_{405} of 0.300 as the cutoff in the IgM EIA, the overall sensitivity of this test is 87% and the specificity is 90%. A higher specificity of 97% at a lower sensitivity (68%) is achieved by regarding A_{405} values exceeding 0.400 as positive. A similar analysis of the IgG EIA showed a sensitivity of 84% and a specificity of 87% when the cutoff A_{405} was 0.400. The acceptance of 0.300 as the cutoff would have increased the sensitivity of the IgG test to 93% at the expense of specificity (69%). In routine diagnoses, we consider an A_{405} ≥0.400 positive and an A_{405} between 0.300 and 0.400 equivocal in both the IgG and the IgM tests.

The above results were based on analyses of both paired and single sera as separate. Next, the practical diagnostic performance of the βVP1 EIA was evaluated on the basis of serodiagnoses in subjects with serum pairs. The occurrence of diagnostic EIA results, positive for IgM or a ≥four-fold rise in the IgG titer, was compared with the occurrence of similar serodiagnoses by the reference test. Groups 1 and 2 together contained 81 subjects with paired samples. By the reference method, diagnoses were obtained for 48 patients, of which 40 (or 45 by considering borderline results diagnostic) also had serodiagnoses by the βVP1 EIA (Fig. 6). Altogether, 40 of 42 diagnoses by the βVP1 EIA were confirmed. To summarize this patient analysis, the recombinant parvovirus antibody EIA had a diagnostic sensitivity of 83% and a specificity of 94%.

The incidence of positive IgM results in the three groups of subjects corresponded well with the clinical classification: 38% in the first group, 12% in the second group, and only 1 of 87 among the laboratory staff were IgM positive by the βVP1 EIA (Fig. 5).

DISCUSSION

We have cloned and expressed the capsid proteins VP1 and VP2 of human parvovirus B19. Several β-galactosidase fusion proteins which together covered the entire capsid were produced in *E. coli*. A major portion (94%) of the

Group 1: ERYTHEMA INFECTIOSUM

B19 IgG capture-RIA

βVP1-EIA	+	+/-	-	total
+	160	4	4	168
+/-	10	1	6	17
-	7	1	16	24
total	177	6	26	209

B19 IgM capture-RIA

βVP1-EIA	+	+/-	-	total
+	76	4	0	80
+/-	22	4	9	35
-	13	8	75	96
total	111	16	84	211

Group 2: RASH OR ARTHRITIS

B19 IgG capture-RIA

βVP1-EIA	+	+/-	-	total
+	40	1	0	41
+/-	9	0	1	10
-	3	2	28	33
total	52	3	29	84

B19 IgM capture-RIA

βVP1-EIA	+	+/-	-	total
+	6	0	4	10
+/-	1	1	0	2
-	3	2	67	72
total	10	3	71	84

Group 3: NONSYMPTOMATIC STAFF

B19 IgG capture-RIA

βVP1-EIA	+	+/-	-	total
+	40	0	3	43
+/-	9	0	9	18
-	9	2	15	26
total	58	2	27	87

B19 IgM capture-RIA

βVP1-EIA	+	+/-	-	total
+	0	0	1	1
+/-	0	2	1	3
-	0	4	79	83
total	0	6	81	87

FIG. 5. Comparison of the βVP1 EIA and the capture RIA. Group 1, sera from school and preschool children with erythema infectiosum-like symptoms and their close contacts; group 2, sera from patients with rashes and/or arthritis; group 3, sera from nonsymptomatic laboratory staff members.

VP1-VP2 protein in recombinant fusion form was nonantigenic. This nonreactive part includes the entire capsid protein VP2. The human antibodies bound in immunoblotting exclusively to fusion proteins with an N-terminal segment of 46 amino acids in VP1. Such a result is surprising considering that VP1 makes up only 4% of the B19 capsid (26). The superiority of VP1 as an antigen also became apparent in a recent study by Brown et al. (7) employing proteins expressed in a baculovirus system. However, other studies have found VP2 of whole viruses to be antigenic (45). It should be added that synthetic peptides encompassing the VP2 region bind human antibodies (14).

Our recombinant antigen (βVP1) was insoluble in physiological conditions. Similar results by other investigators have prevented the direct use of many fusion proteins in EIAs (29, 35), yet the solubility of the present βVP1 antigen in protein-denaturing agents allowed its purification by preparative agarose gel electrophoresis and the subsequent use

SERODIAGNOSES BY PAIRED SAMPLES

capture-RIA

βVP1-EIA	+	+/-	-	total
+	40	0	2	42
+/-	5	0	5	10
-	3	0	26	29
total	48	0	33	81

FIG. 6. Serodiagnoses of all patients of groups 1 and 2 with paired samples. Comparison of the βVP1 EIA and the reference capture RIA. +, positive IgM in either serum or ≥4-fold rise in IgG titer; +/−, equivocal IgM or twofold rise in IgG titer; −, negative IgM and negative or stable IgG titer.

in a solid-phase EIA. The possible trace amounts of SDS in the purified antigen did not prevent antibody binding.

The entire VP1 protein has been previously expressed as a β-galactosidase fusion protein, but the antibody-binding site of this 196-kDa protein remained unmapped (39). Morinet et al. (22) expressed part of VP1-VP2 in fusion with protein A; however, their product was susceptible to degradation. Also, our attempts to express parvoviral proteins in a prokaryotic system in an unfused form (pJLA706) have remained unsuccessful, most likely either because of defective expression or degradation. In general, the production of antigen in fusion with β-galactosidase has the advantage of increased protection against host proteinases. Because of their large sizes, fusion proteins are also easier to separate from the majority of bacterial components. A potential disadvantage is the coexpression of foreign material. However, by using purified recombinant protein as the antigen, our antibody IgM EIA showed an excellent correlation with the clinical grouping of the patients and a good concordance with the reference capture RIA. These results indicate that the βVP1 EIA is suitable for the serodiagnosis of parvovirus B19 infections.

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