JOURNAL OF VIROLOGY, Oct. 1991, p. 5485–5490
0022-538X/91/105485-06$02.00/0

Vol. 65, No. 10

Identification and Mapping of Neutralizing Epitopes of Human Parvovirus B19 by Using Human Antibodies

HIROYUKI SATO,[1]* JOUJI HIRATA,[1] NAOTAKA KURODA,[2] HIROSHI SHIRAKI,[2] YOSHIAKI MAEDA,[2] AND KAZUO OKOCHI[1]

Clinical Laboratory, Kyushu University Hospital, Maidashi 3-1-1, Higashi-ku, Fukuoka 812,[1] and Fukuoka Red Cross Blood Center, 232-11 Kamikoga, Chikushino, Fukuoka 818,[2] Japan

Received 22 April 1991/Accepted 3 July 1991

We identified and mapped the regions responsible for neutralization in the human parvovirus B19 structural protein by using region-specific human antibodies derived from seropositive blood donors. The region-specific antibodies were purified by using affinity columns coupled with synthetic peptides of the hydrophilic regions including the β-turn structure deduced by the predicted secondary structure of VP2. Fifteen highly specific antibodies against the synthetic peptides were obtained. Ten of them were able to precipitate the radiolabeled virus. Six of them proved to be able to protect the colony-forming unit erythroid cells in human bone marrow cell cultures from injury by the virus. The sequences recognized by the six neutralizing antibodies were sites corresponding to amino acids 253 to 272, 309 to 330, 325 to 346, 359 to 382, 449 to 468, and 491 to 515 from the amino-terminal portion of VP2. These observations suggest that the neutralizing epitopes were distributed in the region from amino acid 253 in the amino-terminal portion of VP2 to the carboxyl terminus of VP2.

Human parvovirus B19 has been shown to be the causative agent of erythema infectiosum (fifth disease) in normal children (2) and is also associated with rheumatoid arthritis-like disease in adults (23a, 27a). In patients with hemolytic anemia, it causes aplastic crisis of the bone marrow (11, 18, 22, 23, 26), and in immunosuppressed hosts, it causes persistent pure erythrocyte aplasia (10, 14, 15). An intrauterine infection of the fetus can result in hydrops fetalis and spontaneous abortion in early pregnancy (1, 3, 12). The virion has a single-stranded linear DNA molecule about 5.4 kb long with hairpin structures at each extremity. It contains two major open reading frames, a 71-kDa nonstructural protein encoded by the left open reading frame and overlapping 83-kDa (VP1) and 58-kDa (VP2) structural proteins encoded by the right open reading frame (5, 8, 9, 27) of the B19 genome. The virus can replicate only in the erythroid precursors of the bone marrow (19, 21) and in fetal liver cells (28) in vitro. The production of neutralizing antibodies to the capsid protein was shown to play a major role in limiting parvovirus infection by a colony-forming unit erythroid (CFU-e) assay (6, 7). Previously, we identified one antigenic site corresponding to amino acids 328 to 344 from the amino-terminal portion of the major capsid protein VP2 of the virus using a neutralizing monoclonal antibody named BE11 (24). However, the immunogenicity of the identified site as well as the locations of other sites on the virus that bind neutralizing antibodies have yet to be determined. In this study, we identified the regions in the human parvovirus major structural protein VP2 which will enable the virus to be neutralized with region-specific human antibodies.

MATERIALS AND METHODS

Synthetic peptides. Peptides were synthesized according to their predicted amino acid sequences based on nucleotide sequences of the coding region for the structural protein VP2 of B19 virus (27). The peptides were selected for synthesis from hydrophilic regions including the β-turn structure and bilateral termini by analysis of the hydropathy and the predicted secondary structure of VP2 (4, 16). Seventeen kinds of peptides from 19 to 25 amino acids long were synthesized by a peptide synthesizer (Applied Biosystems, Foster City, Calif.). The purity of individual peptides was examined by reverse-phase high-performance liquid chromatography with an octadecyl (C18) silicated column. The majority of peptides were isolated as a single peak and were used for the enzyme-linked immunosorbent assay (ELISA) and as ligands in affinity chromatography without further purifications.

The peptides were tentatively named S-1 to S-536, with the number representing the first amino acid number of the peptide (Table 1). Their locations and sequences are provided in Fig. 1 and Table 1.

ELISA. The wells of a 96-well microtiter plate (Coster, Cambridge, Mass.) were coated with 500 ng of each synthetic peptide dissolved in 0.01 M $NaHCO_3$ buffer (pH 9.55) at 4°C and left overnight. Unreacted sites on the solid phase were blocked at room temperature for 3 h with 10% goat serum and 0.5% casein in phosphate-buffered saline (PBS) (pH 7.2) containing 0.1% Tween 20. Then, either human plasma samples or antibodies were added to the coated microtiter wells and incubated at 37°C for 1 h after six washings with PBS containing 0.1% Tween 20. The plates were further incubated with goat antibodies to human immunoglobulin G conjugated with horseradish peroxidase (MBL, Nagoya, Japan) at 37°C for 30 min. The plates were washed six times and developed with 150 ml of 0.1% H_2O_2 and 0.04% *ortho*-phenylenediamine in 0.1 M sodium citrate buffer (pH 5.5). The reaction was stopped by the addition of 50 ml of 2.5 M H_2SO_4, and color development was measured in a plate reader (SLT-Labinstruments, Salzburg, Austria) at 492 nm. In a competition ELISA for confirmation, human antibodies preincubated with either 5 mg of synthetic peptides or PBS at 37°C for 30 min were added to microtiter wells coated with the peptide. Antibodies showing a greater than 30% decrease in absorbance in competition ELISA were judged to be positive.

Preparation of human antibodies. A 3-mg sample of each

* Corresponding author.

TABLE 1. Amino acid sequence of synthetic peptides

Peptide	Aminio acids[a]	Peptide sequence[b]
S-1	1–23	MTSVNSAEASTGAGGGGSNSVK
S-40	40–59	FSRQFLIPYDPEHHYKVFSP
S-67	67–86	ASGKEAKVCTISPIMGYSTP
S-128	128–147	VKDVTDKTGGGVQVTDSTTG
S-157	157–176	YKYPYYLGQGQDTLAPELPI
S-185	185–209	AYLTVGDYNTQGISGDSKKLASEES
S-223	223–242	GTGGTASMSYKFPPVPPENL
S-253	253–272	YNPLYGSRLGVPDTLGGDPK
S-288	288–311	FMPGPLVNSYSTKEGDSSNTGAG
S-309	309–330	AGKALTGLSTGTSQNTRISLRP
S-325	325–345	LRPGPVSQPYHHWDTDK
S-340	340–360	WDTDKYVTGINAISHGQTTYG
S-359	359–382	YGNAEDKEYQQGVGRFPNEKEQ
S-449	449–468	LHQPPPQIFLKILPQSGPIG
S-491	491–515	TFKLGPRKATGRWNPQPGVYPPHAA
S-520	520–544	YVLYDPTATDAKQHHRHGYEKPEEL
S-536	536–554	HGYEKPEELWTAKSRVHPL

[a] The numbers represent amino acid numbers in VP2.
[b] Each amino acid is represented by a single-letter code.

peptide was coupled with 0.6 g (dry weight) of cyanogen bromide-activated Sepharose 4B (Pharmacia LKB, Uppsala, Sweden) according to the manufacturer's guide. Human plasma (20 to 50 ml) was applied to the affinity column coupled with each peptide, and the column was washed with PBS. Each antibody that bound itself to the affinity column was eluted by adding a solution containing 3 M sodium thiocyanate and 150 mM NaCl to the column. The eluted antibodies were immediately dialyzed against PBS.

Preparation of virus. B19 virus was prepared from the plasma of a blood donor which reacted to human antiserum against virus B19 by counterimmunoelectrophoresis as described previously (20). B19 virus in the plasma was pelleted by centrifugation at 110,000 × *g* for 2 h (Hitachi RP42 rotor) and purified by centrifugation at 150,000 × *g* for 18 h (Hitachi RSP40T rotor) through a gradient of 20 to 65% sucrose prepared in a buffer containing 20 mM Tris-HCl (pH 7.5), 100 mM NaCl, and 1 mM EDTA. Fractions corresponding to a density of 1.20 to 1.22 g/ml in which B19 virus was detected by counterimmunoelectrophoresis and dot-blot hybridization were pooled.

Radioimmunoprecipitation. The purified virus was radioiodinated by using lactoperoxidase- and glucose oxidase-coupled beads (Bio-Rad Laboratories, Richmond, Calif.) according to the manufacturer's guide. Aliquots of the radiolabeled virus were immunoprecipitated with human sera or affinity-purified antibodies in a buffer containing 150 mM NaCl, 5 mM EDTA, 10 mM Tris-HCl (pH 8.0), and 0.25% gelatin and then incubated with protein A-Sepharose (Pharmacia LKB). After the gel was washed thoroughly in a buffer containing 500 mM NaCl, 5 mM EDTA, 10 mM Tris-HCl (pH 8.0), 0.1% Nonidet P-40, and 1% gelatin, the immune complex was solubilized by boiling in Laemmli's sample buffer and separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis under reducing conditions in Laemmli's discontinuous buffer system (17). The radiolabeled protein in the gel was visualized by autoradiography at −70°C with Kodak XAR film.

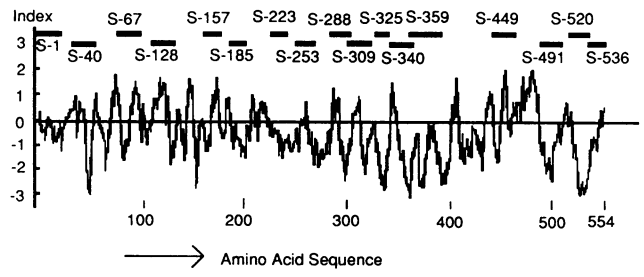

FIG. 1. Hydropathy curve for the polypeptide corresponding to VP2 of human parvovirus B19. The location of each synthetic peptide (S-1 to S-536) is shown.

CFU-e assay. The human plasma samples containing B19 parvoviruses were mixed with diluted monoclonal antibody, human serum from the convalescent phase of erythema infectiosum, or purified human antibodies and incubated at 37°C for 30 min. Bone marrow cells were obtained from a seronegative and hematologically healthy donor with informed consent. Mononuclear cells separated by density gradient centrifugation with Ficoll-Paque (Pharmacia LKB) were incubated with the virus-containing mixture at 4°C for 2 h and then added to a CFU-e assay system (19). CFU-e were assayed in a plasma clot containing 10^5 bone marrow mononuclear cells in 0.5 ml of Iscove's medium (GIBCO Laboratories, Grand Island, N.Y.)–20% fetal calf serum–1% bovine serum albumin (Calbiochem-Behring, La Jolla, Calif.)–10% bovine embryo extract (Flow Laboratories, North Ryde, Australia)–1 U of human erythropoietin (Toyobo, Osaka, Japan) per ml–10% seronegative human AB plasma. The culture was done according to the optimized conditions described in a previous report (24). That is, the final concentrations of the virus-containing plasma, monoclonal antibodies, and human sera were 2%, 1% (10 μg/ml), and 1%, respectively. Affinity-purified human antibodies were used at a final concentration of 0.1 μg/ml. The cultures were evaluated after 7 days of incubation at 37°C with 5% CO_2 in humidified air. After the clot was fixed and stained, the numbers of CFU-e, defined as colonies of eight or more benzidine-positive cells, were counted. All experiments were done in replicate plates.

RESULTS

Seventeen kinds of peptides consisting of from 19 to 25 amino acids, which covered 59% of VP2, were synthesized by a peptide synthesizer. Sixty-one human plasma samples which had antibodies against virus B19 were selected from the blood donors by countercurrent immunoelectrophoresis, using the same virus as the antigen that was used in the CFU-e protection assay described below. The reactivity of the synthetic peptides with the selected plasma samples was examined by an ELISA (Fig. 2). Two (S-128 and S-340) of 17 peptides did not react with any of the human plasma samples. Each human plasma sample which gave the highest titer of antibody against each peptide was applied to an immunoadsorbent column coupled with each synthetic peptide. The region-specific antibodies were eluted by affinity column chromatography. The antibodies were named Ab-1 to Ab-536, each having the same code number as the peptide that was used for purification. The specificities of these antibodies were examined by ELISA. As shown in Table 2, no cross-reactivity was found between the peptides. In addition, the specificity of each antibody was confirmed by competition ELISA. However, one seronegative plasma sample did not give a positive reaction with any of the peptides. The reactivities of these 15 antibodies with virus

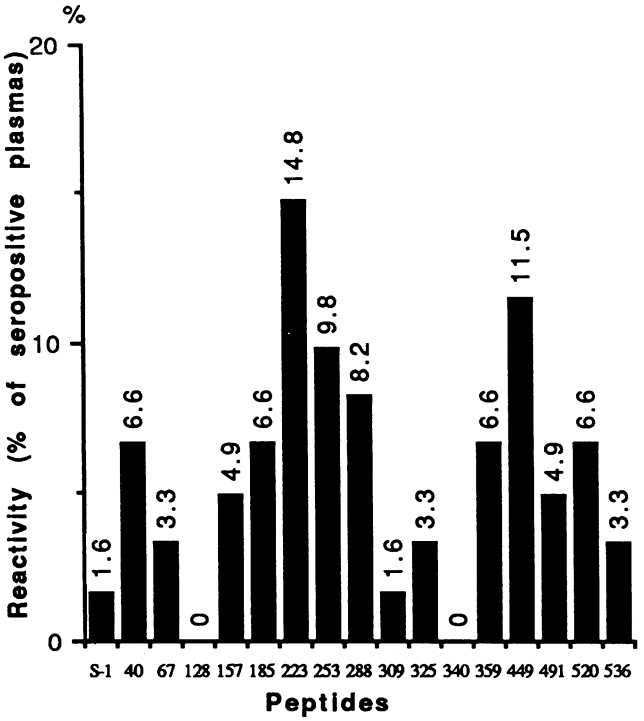

FIG. 2. Seroreactivity of plasma specimens from 61 seropositive blood donors with synthetic peptides. Specimens showing a greater than 30% decrease in absorbance in competition ELISA were judged to be positive.

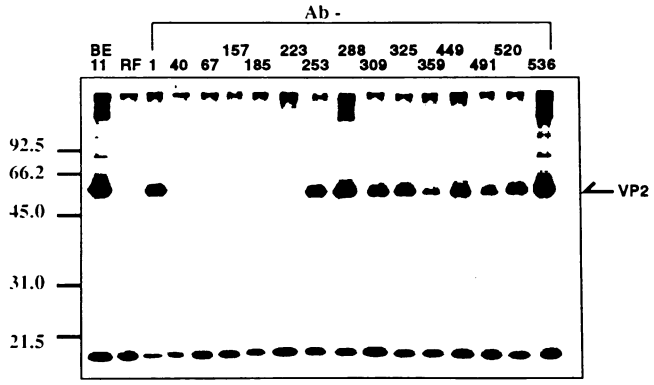

FIG. 3. Immunoprecipitation of radioiodinated B19 virus by various antibodies. The labeled virion was immunoprecipitated with the monoclonal antibody against B19 virus (BE11), with monoclonal antibody RF465 against p19 of the human T-lymphotropic virus type I *gag* gene product (RF), or with each antibody purified on an affinity column fixed with peptides representing various regions of the major viral capsid protein VP2 (Ab-1 to Ab-536). The markers on the left indicate the sizes (in kilodaltons) of standard proteins run in parallel.

B19 were tested by radioimmunoprecipitation with ^{125}I-labeled virions (Fig. 3). Ten of them (Ab-1, Ab-253, Ab-288, Ab-309, Ab-325, Ab-359, Ab-449, Ab-491, Ab-520, and Ab-536) could precipitate the virus. A 58-kDa protein corresponding to VP2, a major structural protein of virus B19 was also observed. However, five antibodies (Ab-40, Ab-67, Ab-157, Ab-182, and Ab-223) which reacted with the peptides corresponding to amino acids 40 to 242 from the amino-terminal portion of VP2 were not able to precipitate the virus.

The neutralizing activities of these antibodies were examined for their protective effect against injury of late erythroid progenitor cells (CFU-e) in the bone marrow by B19 virus in vitro. Human bone marrow cells were cultured with the virus preincubated with each antibody. The protecting effect of each antibody was determined by the relative activity of

TABLE 2. Reactivity of affinity-purified antibodies with each peptide (ELISA)[a]

Peptide	Affinity-purified antibody															Seronegative plasma (% inhibition)[b]
	1	40	67	157	185	223	253	288	309	325	359	449	491	520	536	
S-1	2.083	0.000	0.022	0.008	0.077	0.009	0.014	0.002	0.001	0.005	0.033	0.019	0.021	0.012	0.015	0.097 (0.0)
S-40	0.002	2.855	0.013	0.000	0.000	0.000	0.006	0.002	0.000	0.006	0.013	0.009	0.001	0.003	0.005	0.074 (0.0)
S-67	0.007	0.012	0.538	0.009	0.003	0.005	0.006	0.003	0.000	0.009	0.013	0.011	0.004	0.004	0.012	0.044 (0.0)
S-128	0.018	0.008	0.049	0.000	0.003	0.004	0.005	0.004	0.000	0.027	0.029	0.027	0.058	0.038	0.019	0.212 (0.0)
S-157	0.009	0.003	0.022	1.885	0.003	0.003	0.008	0.030	0.000	0.007	0.010	0.019	0.033	0.015	0.051	0.031 (0.0)
S-185	0.003	0.003	0.032	0.019	1.438	0.000	0.014	0.016	0.001	0.024	0.031	0.052	0.077	0.019	0.043	0.021 (0.0)
S-223	0.007	0.000	0.027	0.063	0.003	0.883	0.006	0.000	0.000	0.015	0.021	0.015	0.017	0.013	0.020	0.073 (1.4)
S-253	0.004	0.000	0.016	0.010	0.003	0.006	1.127	0.006	0.021	0.024	0.018	0.012	0.010	0.009	0.013	0.060 (0.0)
S-288	0.007	0.001	0.027	0.011	0.009	0.007	0.007	0.747	0.000	0.015	0.016	0.012	0.005	0.000	0.009	0.033 (0.0)
S-309	0.012	0.009	0.054	0.014	0.013	0.010	0.000	0.000	0.256	0.001	0.015	0.005	0.005	0.003	0.016	0.031 (0.0)
S-325	0.021	0.006	0.035	0.019	0.011	0.014	0.004	0.000	0.000	0.486	0.017	0.011	0.031	0.011	0.046	0.057 (0.0)
S-340	0.009	0.002	0.030	0.012	0.006	0.011	0.012	0.014	0.004	0.015	0.068	0.007	0.011	0.006	0.017	0.062 (0.0)
S-359	0.008	0.003	0.023	0.025	0.016	0.012	0.012	0.007	0.005	0.017	0.786	0.002	0.005	0.001	0.006	0.039 (3.8)
S-449	0.006	0.000	0.020	0.019	0.000	0.004	0.012	0.010	0.004	0.047	0.011	1.575	0.011	0.003	0.013	0.025 (0.0)
S-491	0.011	0.012	0.025	0.023	0.013	0.009	0.012	0.016	0.001	0.025	0.003	0.002	2.533	0.002	0.010	0.054 (0.0)
S-520	0.010	0.003	0.026	0.020	0.012	0.018	0.010	0.006	0.002	0.024	0.014	0.008	0.014	0.854	0.019	0.085 (0.0)
S-536	0.034	0.009	0.037	0.020	0.011	0.007	0.012	0.015	0.006	0.026	0.009	0.005	0.014	0.072	0.396	0.070 (0.0)
% Inhibition[c]	65.1	98.7	51.7	62.4	84.9	34.6	94.3	83.2	66.1	99.2	97.5	98.8	92.1	96.7	94.6	

[a] A_{492} is shown. Reaction with the peptide used for purification is underlined.
[b] Inhibition rate in the reaction of seronegative plasma with each peptide.
[c] Inhibition rate in the reaction of each antibody with the peptide used for purification.

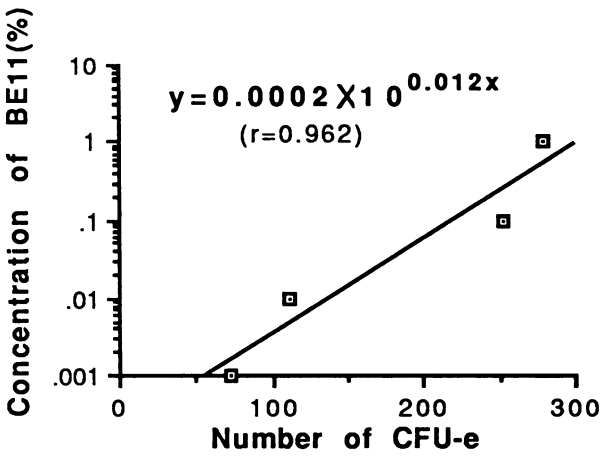

FIG. 4. BE11 protects CFU-e from injury by B19 virus. Human plasma specimens containing B19 parvoviruses were mixed with serially diluted monoclonal antibody BE11 at 37°C for 30 min and were further incubated with bone marrow cells at 4°C for 2 h and then added to the CFU-e assay system. The mean values of the CFU-e count were plotted against the logarithm of the concentration of BE11, and the putative regression line is shown.

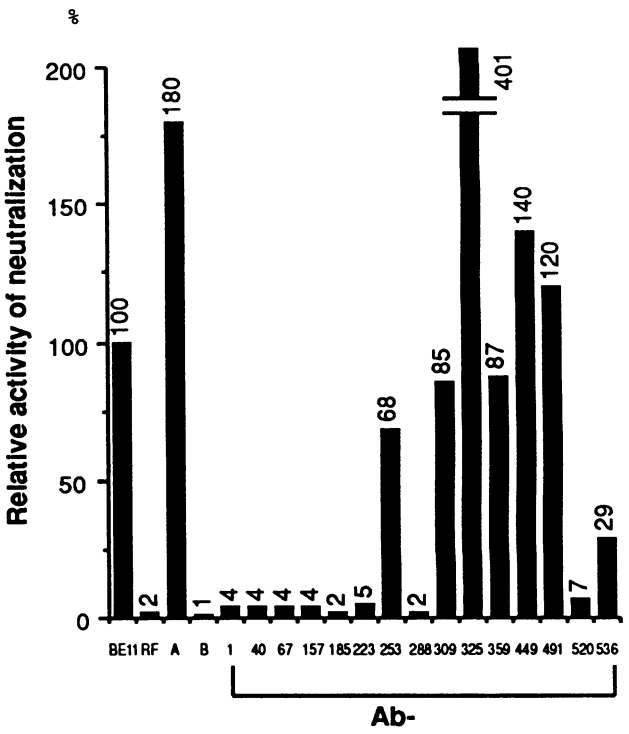

FIG. 5. Relative activities of neutralization. BE11, RF465 (RF), human serum from the convalescent phase of erythema infectiosum (A), human serum before infection of erythema infectiosum (B), or affinity-purified antibodies (Ab-1 to Ab-536) were preincubated with the virus-containing plasma at 37°C for 30 min and then were further incubated with bone marrow cells at 4°C for 2 h and cultured in a plasma clot in the presence of erythropoietin at 37°C for 7 days. The relative activity of neutralization of each antibody was calculated from the regression line (shown in Fig. 4) as a concentration of BE11 and estimated in comparison with the activity of BE11 at a rate of 100%.

neutralization in comparison with that of BE11. Serially diluted BE11 was added to the CFU-e assay in the presence of B19 virus, and the mean values of the CFU-e count were plotted against the logarithm of the concentration of BE11. The curve obtained indicated a straight-line relationship (Fig. 4). The relative activity of neutralization of each antibody was calculated from the regression line as a concentration of BE11 and estimated in comparison with the activity of BE11 at a rate of 100% (Fig. 5). Six antibodies (Ab-253, Ab-309, Ab-325, Ab-359, Ab-449, and Ab-491) showed more than 50% activity for protecting CFU-e from injury by the virus. Ab-536, the antibody against the carboxyl terminus, had 29% activity. The other human antibodies had no any significant activity, nor did control ascites fluid RF465, which is a monoclonal antibody against a *gag* gene product of human T-lymphotropic virus type I, or a seronegative human serum used as a negative control (25).

DISCUSSION

Neutralizing antibodies and their binding sites on several kinds of virus were identified and analyzed by using monoclonal antibodies. This requires many kinds of sequence-recognizing monoclonal antibodies against viral capsid proteins or envelope proteins to cover the many neutralizing epitopes. Furthermore, it is not easy to evaluate whether a candidate for a vaccine which is deduced by studies using animal antibodies is effective for humans, particularly in regard to the viruses which have a limited host range or are highly pathogenic for human beings.

The production of neutralizing antibodies to capsid proteins has been shown by a CFU-e assay to play a major role in limiting parvovirus infection (13, 19). Previously, we identified one site corresponding to amino acids 328 to 344 from the amino-terminal portion of the major capsid protein VP2 of the virus by using a neutralizing monoclonal antibody named BE11 (24). In this study, we attempted to identify and map the neutralizing epitopes of human parvovirus B19 using amino acid sequence-recognizing antibodies of human origin.

Among the 17 kinds of synthetic peptides, we could not find any peptide which reacted with all of the seropositive human plasma samples defined by countercurrent immunoelectrophoresis. However, 15 of 17 peptides reacted with some of the seropositive plasma samples by ELISA. Then the amino acid sequence-recognizing antibodies were eluted by affinity column chromatography. The eluted antibodies were highly specific, and no cross-reactivities were observed. It is thus suggested that 15 regions of VP2 contained antigenic determinants for the human immune system. By radioimmunoprecipitation, 10 antibodies were found to precipitate the virus, while 5 antibodies, which reacted with the peptides corresponding to amino acids 40 to 242 from the amino-terminal portion of VP2, did not do so. We suggest that the sequence of each peptide to which the 10 antibodies reacted was on the surface of the virion. As there is very little variation among B19 virus isolates and the regions which the antibodies did not react to were nested in the amino-terminal half of VP2, we suggest that the antigenic determinant of the five antibodies is not exposed on the surface of the virion (the so-called hidden epitopes).

The neutralizing activities of these antibodies were also examined for their protective effect against injury of late erythroid progenitor cells (CFU-e) in the bone marrow by B19 virus in vitro. Since the hemoglobin levels of the

seropositive donors were high enough for blood donation, antibodies against B19 virus might not affect CFU-e in vivo, and the antibodies themselves did not affect the CFU-e count in vitro (data not shown). Human bone marrow cells were cultured with virus that was preincubated with each antibody. The protecting effect of each antibody was determined by the relative activity of neutralization in comparison with that of BE11. To test whether the purified antibody caused an aggregation of the B19 virion or not, we examined the lattice formation of the virion by each antibody in agarose gel using the Ouchterlony double-diffusion technique and counterimmunoelectrophoresis in various antigen/antibody ratios. No immunoprecipitation could be observed by either method (data not shown). These findings suggested that the CFU-e escaped virus infection by means of neutralization rather than aggregation of the virus by each antibody. Six antibodies showed more than 65% activity, while Ab-536, an antibody against the carboxyl terminal of VP2, showed very little activity. In particular, Ab-325 had about four times the activity of BE11. Three antibodies (Ab-1, Ab-288, and Ab-520) which were able to bind to the virus by immunoprecipitation did not protect CFU-e from injury by the virus. All five antibodies which did not react with the virus by immunoprecipitation also showed no protective effect. The neutralizing epitopes were detected and distributed from the region corresponding to amino acid 253 in the amino-terminal portion to the carboxyl terminal of VP2. No neutralizing epitopes were found in the amino-terminal half of VP2 as far as was tested. These observations suggest that the region in the carboxyl-terminal half of VP2 is highly associated with neutralization of virus B19. However, direct evidence that this region of VP2 adheres to the cellular membrane at a specific receptor has not yet been obtained. As seropositive human plasmas are thought to contain polyclonal antibodies, there might be another neutralizing antibody which recognizes a conformational or sequential structure other than that of the peptides tested. Further study of the region of VP1 that does not share an amino acid sequence with VP2 is necessary. The peptides, including the neutralizing epitopes, are considered to be useful tools for the analysis of the cellular receptor. The region containing the neutralizing epitopes, as defined by human serum samples, should be included in the protein used as a vaccine. Furthermore, the neutralizing antibodies eluted by synthetic peptides could be used as a passive immunization for therapeutic use.

Our approach is thus able to provide information regarding the neutralizing epitopes and their immunogenicity, as well as provide a method for purifying the neutralizing antibodies in high titers for passive immunization. This approach should be applicable to many virus diseases in which neutralizing antibodies play a major role in excluding virus infections of humans and animals.

ACKNOWLEDGMENTS

We thank S. Narayanan of New York Medical College and B. Quinn of Kyushu University for critical reading of the manuscript.

This work was supported by a Grant-in-Aid for Science Research (C) (63570212) from the Ministry of Education, Science and Culture of Japan.

REFERENCES

1. **Anand, A., E. S. Gray, T. Brown, J. P. Clewley, and B. J. Cohen.** 1987. Human parvovirus infection in pregnancy and hydrops fetalis. N. Engl. J. Med. **316**:183–186.
2. **Anderson, M. J., S. E. Jones, S. P. Fisher-Hoch, E. Lewis, S. M. Hall, C. L. Bartlett, B. J. Cohen, P. P. Mortimer, and M. S. Pereira.** 1983. The human parvovirus, the cause of erythema infectiosum (fifth disease)? Lancet **i**:1378.
3. **Brown, T., A. Anand, L. D. Ritchie, J. P. Clewley, and T. M. S. Reid.** 1984. Intrauterine parvovirus infection associated with hydrops fetalis. Lancet **ii**:1033–1034.
4. **Chou, P. Y., and G. D. Fasman.** 1974. Prediction of protein conformation. Biochemistry **13**:222–244.
5. **Clewley, J. P.** 1984. Biochemical characterization of a human parvovirus. J. Gen. Virol. **65**:241–245.
6. **Cohen, B. J., P. P. Mortimer, and M. S. Pereira.** 1983. Diagnostic assay with monoclonal antibodies for the human serum parvovirus-like virus (SPLV). J. Hyg. **91**:113–130.
7. **Cossart, Y. E., B. Cant, A. M. Field, and D. Widdows.** 1975. Parvovirus-like particles in human serum. Lancet **i**:72–73.
8. **Cotmore, S. F., V. C. Mckie, L. J. Anderson, C. R. Astell, and P. Tattersall.** 1986. Identification of the major structural and nonstructural proteins encoded by human parvovirus B19 and mapping of their genes by procaryotic expression of isolated genomic fragments. J. Virol. **60**:548–557.
9. **Cotmore, S. F., and P. Tattersall.** 1984. Characterization and molecular cloning of a human parvovirus genome. Science **226**:1161–1165.
10. **Coulombel, L., F. Morinet, and G. Tchernia.** 1989. Parvovirus infection, leukaemia, and immunodeficiency. Lancet **i**:101–102.
11. **Duncan, J. R., C. B. Potter, M. D. Cappelini, J. B. Kurtz, M. J. Anderson, and D. J. Weatherall.** 1983. Aplastic crisis due to parvovirus infection in pyruvate kinase deficiency. Lancet **ii**: 14–16.
12. **Knott, P. D., G. A. C. Welply, and M. J. Anderson.** 1984. Serologically proved intrauterine infection with parvovirus. Br. Med. J. **289**:1660.
13. **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.
14. **Kurtzman, G. J., B. Cohen, P. Meyers, A. Amunullah, and N. S. Young.** 1988. Persistent B19 parvovirus infection as a cause of severe chronic anaemia in children with acute lymphocytic leukemia. Lancet **ii**:1159–1162.
15. **Kurtzman, G. J., K. Ozawa, B. Cohen, G. Hanson, R. Oseas, and N. S. Young.** 1987. Chronic bone marrow failure due to persistent B19 parvovirus infection. N. Engl. J. Med. **317**:287–294.
16. **Kyte, J., and R. F. Doolittle.** 1982. A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. **157**:105–132.
17. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
18. **Mortimer, P. P.** 1983. Hypothesis: the aplastic crisis of hereditary spherocytosis is due to a single transmissible agent. J. Clin. Pathol. **36**:445–448.
19. **Mortimer, P. P., R. K. Humphries, J. G. Moore, R. H. Purcell, and N. S. Young.** 1983. A human parvovirus-like virus inhibits haemopoietic colony formation in vitro. Nature (London) **302**: 426–429.
20. **Okochi, K., R. Mori, M. Miyazaki, B. J. Cohen, and P. P. Mortimer.** 1984. Nakatani antigen and human parvovirus (B19). Lancet **i**:160–161.
21. **Ozawa, K., J. Ayub, H. Yu-shu, G. Kurtzman, T. Shimada, and N. Young.** 1987. Novel transcription map for the B19 (human) pathogenic parvovirus. J. Virol. **61**:2395–2406.
22. **Pattison, J. R., S. E. Jones, J. Hodgson, L. R. Davis, J. M. White, C. E. Stroud, and L. Murtaza.** 1981. Parvovirus infection and hypoplastic crisis in sickle-cell anemia. Lancet **i**:664–665.
23. **Rao, K. R., A. R. Patel, M. J. Anderson, J. Hodgson, S. E. Jones, and J. R. Pattison.** 1983. Infection with parvovirus-like virus and aplastic crisis in chronic hemolytic anemia. Ann. Intern. Med. **98**:930–932.

23a. **Reid, D. M., T. Brown, T. M. S. Reid, J. A. N. Rennie, and C. J. Eastmond.** 1985. Human parvovirus-associated arthritis: a clinical and laboratory description. Lancet **ii**:422–425.

24. **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.
25. **Sato, H., and K. Okochi.** 1986. Transmission of human T-cell leukemia virus (HTLV-I) by blood transfusion: demonstration of proviral DNA in recipients' blood lymphocytes. Int. J. Cancer **37**:395–400.
26. **Sergeant, G. R., J. M. Topley, K. Mason, B. E. Sergeant, J. R. Pattison, S. E. Jones, and R. Mohamed.** 1981. Outbreak of aplastic crisis in sickle-cell anemia associated with parvovirus-like agent. Lancet **ii**:695–698.
27. **Shade, R. O., M. C. Blundell, S. S. 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.
27a. **White, D. G., P. P. Mortimer, D. R. Blake, A. D. Woolf, B. J. Cohen, and P. A. Bacon.** 1985. Human parvovirus arthropathy. Lancet **ii**:419–421.
28. **Yaegashi, N., H. Shiraishi, T. Takeshita, M. Nakamura, A. Yajima, and K. Sugamura.** 1989. Propagation of human parvovirus B19 in primary culture of erythroid lineage cells derived from fetal liver. J. Virol. **63**:2422–2426.