JOURNAL OF VIROLOGY, Mar. 1999, p. 1974–1979 Vol. 73, No. 3
0022-538X/99/$04.00+0

Generation of Neutralizing Human Monoclonal Antibodies against Parvovirus B19 Proteins

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Received 10 September 1998/Accepted 9 December 1998

Infections caused by human parvovirus B19 are known to be controlled mainly by neutralizing antibodies. To analyze the immune reaction against parvovirus B19 proteins, four cell lines secreting human immunoglobulin G monoclonal antibodies (MAbs) were generated from two healthy donors and one human immunodeficiency virus type 1-seropositive individual with high serum titers against parvovirus. One MAb is specific for nonstructural protein NS1 (MAb 1424), two MAbs are specific for the unique region of minor capsid protein VP1 (MAbs 1418-1 and 1418-16), and one MAb is directed to major capsid protein VP2 (MAb 860-55D). Two MAbs, 1418-1 and 1418-16, which were generated from the same individual have identity in the cDNA sequences encoding the variable domains, with the exception of four base pairs resulting in only one amino acid change in the light chain. The NS1- and VP1-specific MAbs interact with linear epitopes, whereas the recognized epitope in VP2 is conformational. The MAbs specific for the structural proteins display strong virus-neutralizing activity. The VP1- and VP2-specific MAbs have the capacity to neutralize 50% of infectious parvovirus B19 in vitro at 0.08 and 0.73 μg/ml, respectively, demonstrating the importance of such antibodies in the clearance of B19 viremia. The NS1-specific MAb mediated weak neutralizing activity and required 47.7 μg/ml for 50% neutralization. The human MAbs with potent neutralizing activity could be used for immunotherapy of chronically B19 virus-infected individuals and acutely infected pregnant women. Furthermore, the knowledge gained regarding epitopes which induce strongly neutralizing antibodies may be important for vaccine development.

Parvovirus B19 is an autonomous member of the family of *Parvoviridae* and the only human pathogenic parvovirus described so far. Discovered in 1975 (7), parvovirus B19 was subsequently identified as the causative agent of the common childhood disease erythema infectiosum (1), a usually benign illness which is associated with fever and a characteristic rash. Under specific circumstances, B19 virus infections can provoke a variety of additional, more severe clinical symptoms. Frequent complications occur in patients with underlying hemolytic disorders which show a strong tendency to develop aplastic crises (26). In utero infection can cause hydrops fetalis and fetal death (5). Furthermore, acute polyarthralgia and arthritis are frequently associated with parvovirus B19 infections, particularly in adult women (22, 31).

In immunocompetent hosts, B19 viremia is usually rapidly cleared and followed by the production of specific antibodies to structural proteins VP1 and VP2, whereas in immunocompromised patients, viral persistence is frequently observed (17, 21). The detection of VP1- and VP2-specific antibodies is the basis for the diagnosis of acute or past B19 virus infections. In addition, antibodies against nonstructural protein NS1 may have utility as an indicator of chronic or persistent forms of B19 virus infections with delayed virus elimination (32, 33). Together with the destruction of the erythroid target cells, antiviral antibodies appear to be most important for recovery from a parvovirus B19 infection. Therefore, persons suffering from persistent B19 virus infection, e.g., immunosuppressed patients, are successfully treated with immunoglobulin preparations containing B19 virus-specific antibodies (10). However, the use of human monoclonal antibodies (MAbs) against B19 virus should be more expedient because of their better availability, higher specificity, and reduced contamination risk. At the time this study was started, murine MAbs against structural proteins VP1 and VP2 (3, 34) and two human MAbs against VP2 had been described (2). MAbs against the NS1 protein have not been produced yet. In the present study, human MAbs were generated by using antibody-producing B lymphocytes isolated from two healthy individuals with past B19 virus infections and one human immunodeficiency virus (HIV-1)-seropositive individual. Four cell lines secreting human immunoglobulin G (IgG) were obtained with specificity for the unique region of minor capsid protein VP1, major structural protein VP2, and nonstructural protein NS1. The immunochemical properties of these human MAbs were characterized together with their respective virus-neutralizing capacities, and their epitopes were mapped. These antibodies may prove to be important reagents for the therapy of B19 virus-infected pregnant women or chronically infected patients. Furthermore, they may represent ideal tools for studying the pathogenesis of B19 virus infection in vitro and allow new insights into the immunogenicity of B19 virus proteins and for vaccine development.

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MATERIALS AND METHODS

Serum and blood samples. Serum and heparinized blood samples were derived from healthy volunteers and from HIV-1-seropositive individuals seen as inpatients at the VA Medical Center. All sera were tested for the presence of antibodies to B19 virus proteins by a noncommercial enzyme-linked immunosorbent assay (ELISA).

Recombinant proteins and peptides. The NS1 and VP1 genes and their subfragments were linked with a nucleotide sequence coding for a polyhistidine stretch of amino acids (His tag). Subsequently, recombinant proteins were expressed in *Escherichia coli* with this His tag attached at the amino-terminal end. The NS1 protein comprises the full length of the open reading frame (671 amino acids), and the unique part of minor capsid protein VP1 contains 226 amino acids. The proteins were purified by affinity chromatography via the polyhistidine stretch by using an Ni^{2+} matrix followed by preparative sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The subfragments of the VP1 unique region fused to a His tag were used without prior purification in a modified ELISA (see below). The VP2 protein was produced in the form of self-assembled recombinant empty capsids in a baculovirus expression system as previously described (15).

For fine mapping of the epitopes, several overlapping synthetic peptides were designed by using the published parvovirus B19 genome sequence (27) and synthesized by using the 9-fluorenylmethoxycarbonyl strategy and a Milligen 9050 Pep Synthetsizer (13).

Binding assays. Screening of sera for the presence of B19 virus-specific antibodies, binding of MAbs to recombinant proteins and synthetic peptides, and epitope mapping were performed by ELISA. Microtiter plates were coated with recombinant proteins (100 ng per well) or peptides (500 ng per well) dissolved in 0.2 M carbonate buffer (pH 9.5) at 4°C overnight. Plates were blocked for 1 h at 37°C with 2% bovine serum albumin in phosphate-buffered saline (PBS), washed three times with PBS containing 0.05% Tween 20, and then incubated for 1.5 h at 37°C with diluted sera or MAbs. After washing, the binding of antibodies was detected with alkaline phosphatase-conjugated goat anti-human IgG (Fc specific; Zymed, San Francisco, Calif.) diluted 1:1,000 in PBS–0.05% Tween 20 for another 1.5 h at 37°C. The plates were washed again, the substrate *p*-nitrophenyl phosphatase in 10% diethanolamine (Sigma, St. Louis, Mo.) was added and the mixture was incubated for 30 min. The A_{410} was read in an ELISA reader. Sera from individuals without a prior B19 virus infection and human MAb (670-D), specific for conserved region 5 of membrane protein gp120 of HIV-1 (35), were used as negative controls. The cutoff level was calculated as the mean optical density plus three standard deviations obtained from three negative controls.

The subfragments of the VP1 unique region were tested in a modified ELISA. The microtiter plates were coated with a murine MAb directed against the polyhistidine sequence to capture the His-tagged subfragments of the VP1 unique region from crude bacterial extracts. After washing, recombinant proteins with polyhistidine sequences were allowed to adsorb to the wells and exposed for antibody binding in a standard ELISA (Qiagen, Hilden, Germany).

VP2-specific IgM was determined by using a commercial parvovirus B19 IgM capture immunoassay (Biotrin International, Dublin, Ireland) in accordance with the manufacturer's protocol.

Production of heterohybridoma cell lines secreting MAbs against B19 virus proteins. Human MAbs were produced as previously described (12). Briefly, peripheral blood mononuclear cells were purified from a heparinized blood sample by gradient centrifugation on Histopaque (Sigma) and subsequently infected with Epstein-Barr virus. The cells were cultured for 3 to 4 weeks, and their supernatants were screened for IgG antibodies against B19 virus recombinant proteins by ELISA. Positive cell populations were expanded in culture for an additional week and fused with mouse × human heteromyeloma cell line SHM-D33 (30) in order to stabilize the antibody-producing cells. Hybridomas producing B19 virus-specific antibodies were cloned by limiting dilutions until monoclonality was achieved.

Purification and characterization of MAbs. Human MAbs were purified from culture supernatants by using HiTrap Protein G columns (Pharmacia, Uppsala, Sweden) in accordance with the manufacturer's protocol. IgG concentration was determined by ELISA using microtiter plates coated with goat anti-human IgG (γ specific; Organon Teknika, Durham, N.C.) and then incubated with antibody preparations. Bound IgG was detected with alkaline phosphatase-conjugated goat anti-human IgG (γ specific; Zymed). Affinity-purified human IgG (Organon Teknika) was used for the standard curve.

Determination of the IgG subclass and the subtypes of light chains of the MAbs was performed by ELISA. Plates were coated with an unlabeled murine MAb against human subclasses IgG1 to IgG4 or with a polyclonal rabbit antibody against the human κ or λ chain (Sigma). Bound human IgG was detected with alkaline phosphatase-labeled goat anti-human IgG (Zymed).

Virus neutralization assay. The ability of the MAbs to neutralize parvovirus B19 was determined by using a human erythroid-cell colony-forming assay (15). Parvovirus B19-containing sera from patients with acute infection and a B19-negative human serum, used as a control, were incubated at 56°C for 30 min for complement inactivation. The highest serum dilution at which colony formation was completely inhibited was used in subsequent experiments. Sera and human MAbs were diluted in Iscove's modified Dulbecco medium (Gibco Life Technologies) containing 20% fetal bovine serum (Alanta Biologicals) and supplemented with 2% penicillin-streptomycin and 2% glutamine (IMDM). Serial 10-fold dilutions of purified human MAbs in IMDM (50 μl) were incubated for 2 h at 4°C with 30 μl of diluted human serum containing parvovirus. The MAb-virus mixture was then incubated with normal bone marrow mononuclear cells at 2×10^6 cells/ml (final concentration, 3×10^5 cells) in a volume of 150 μl for 2 h at 4°C. The cells were suspended in medium containing 0.8% methylcellulose (Stem Cell Technologies) supplemented with recombinant erythropoietin (final concentration, 5 U/ml; Ortho Biotech Inc.) and incubated in a six-well plate at 37°C for 7 days. The number of erythroid-cell colonies per well was determined by microscopic evaluation. All assays were done in duplicate. Controls included bone marrow cells incubated without serum or virus and with virus with medium only and an irrelevant human MAb, 670-D, specific for gp120 of HIV-1.

TABLE 1. Serological data of the individuals from whom MAbs were derived[a]

Individual	IgG to NS1	IgG to VP1	IgM to VP1/2	B19 virus DNA	HIV-1
1418	−	+	−	−	−
1424	+	+	+	−	−
860	−	+	−	−	+

[a] +, present; −, absent.

Sequence determination of the human MAb variable domains. For isolation of total RNAs from the hybridoma cell lines, a commercially available purification system was applied (RNeasy; Qiagen GmbH, Hilden, Federal Republic of Germany). Reverse transcription-PCR was done by using total RNA preparations and oligonucleotides as primers, located in conserved regions of the IgG light and heavy chains, in order to amplify the regions encoding the human MAb variable domains (Titan Kit; Boehringer Mannheim GmbH, Mannheim, Federal Republic of Germany). The primer sequences used for reverse transcription-PCR were selected as described previously (19, 29). Since the different subtypes of the heavy and light chains cannot be amplified with single primer sets, it was necessary to perform several PCRs with different primer combinations. For amplification, the following conditions were used: 30 min at 50°C (reverse transcription), 2 min at 94°C, 30 s at 94°C (denaturation), 45 s at 55°C (hybridization), and 60 s at 68°C (polymerization). PCR cycles, starting with the denaturation step, were repeated 35 times and followed by incubation for 7 min at 68°C. PCR products were directly sequenced by using the Dideoxy-Termination Cycle Sequencing Kit and analyzed with an automated DNA sequence analyzer (Applied Biosystems, Weiterstadt, Federal Republic of Germany). Both strands of the PCR products were sequenced by using the primers applied for amplification. The whole procedure was done at least two times in order to exclude mutations which may have been caused by PCR.

Nested PCR for detection of B19 virus genomes. Detection of B19 virus genomes by nested PCR was performed as previously described (14). Briefly, serum samples were diluted 1:1 with water and incubated at 95°C for 10 min. Aggregated proteins were removed by centrifugation, and a 2-μl volume of the supernatants was used in the first cycling reaction. In the second round, 2 μl of the first amplification reaction was applied. The primers used were homologous to the following positions of the published sequence of isolate pYT103 (27): 2901 to 2918 (forward) and 3511 to 3895 (reverse) in the first round and 2956 to 2972 (forward) and 3431 to 3448 (reverse) in the second round. The PCR parameters were 1 min at 95°C (denaturation), 45 s at 94°C (hybridization), and 90 s at 72°C (polymerization). Forty PCR cycles were performed. Identical conditions were used for the first and second rounds of amplification. Ten microliters of each reaction was separated on a 2% agarose gel and then stained with ethidium bromide. A band with a molecular size of approximately 500 bp indicated a positive result.

Nucleotide sequence accession numbers. The nucleotide sequence data from this study have been deposited in the GenBank database under accession no. AF09498 to AF092503.

RESULTS

Generation of human MAbs against parvovirus B19. Peripheral blood mononuclear cells from normal, healthy individuals with high titers of serum antibodies against B19 virus proteins were used for generation of MAbs. Individual 1418, from whom cell lines 1418-1 and 1418-16 were obtained, had no clinical or laboratory evidence of an acute or chronic B19 virus infection (Table 1). B19 virus genomes could not be detected in the serum by nested PCR. In addition, the serum was negative for B19 virus-specific IgM, and the individual had no symptoms correlated to B19 virus infections for at least 6

TABLE 2. Characteristics of the human MAbs generated in this study

Human MAb	Specificity	IgG subclass	Light chain	Family	
				Heavy chain	Light chain
1418-1	VP1 unique region	1	κ	V_H1	$V_κ3$
1418-16	VP1 unique region	1	κ	V_H1	$V_κ3$
1424	NS1	3	λ	V_H3	$V_λ1$
860-55	VP2	3	λ	ND[a]	ND

[a] ND, not done.

months before the blood sample was obtained. The individual from whom cell line 1424 was derived showed low titers of VP1-specific IgM but was negative for the presence of B19 virus genomes by nested PCR. In addition, this person was also free of symptoms. Therefore, it may be assumed that this individual was in the convalescent phase after parvovirus B19 infection at the time when the blood sample was obtained. MAb 860-55D was produced from cells derived from an HIV-1-positive individual whose blood contained serum IgG antibodies against B19 virus proteins. He had no signs of acute or chronic B19 virus infection, and furthermore, no B19 virus genome could be detected by nested PCR and the serum was negative for B19 virus-specific IgM.

All four hybridomas, 1418-1, 1418-16, 1424, and 860-55D, were stabilized by sequential cloning using limiting dilutions. Monoclonality was determined by double final cloning at 1 cell per well. Protein G-purified MAbs analyzed by SDS-PAGE and stained with $AgNO_3$ showed two separate bands for the heavy and light chains (data not shown). The IgG subclasses and light-chain types were determined by ELISA (Table 2). Both VP1-specific MAbs 1418-1 and 1418-16 were assigned to subclass IgG1 and light chain κ. MAb 1424 against the NS1 protein displayed IgG3 and λ-chain characteristics, whereas 860-55D showed IgG1 and λ-chain features.

Epitope mapping. In order to define the antigenic domain recognized by NS1-specific human MAb 1424, four partly overlapping fragments of the NS1 protein were used as antigens in an ELISA. The protein fragments were produced in *E. coli* and purified by affinity chromatography and preparative SDS-PAGE as described above. Human MAb 1424 reacted exclusively with fragment NS/D, which includes the region between amino acids 493 and 671 of the NS1 protein (Fig. 1). To further localize the epitope, 17 synthetic peptides that had an average length of 18 residues and overlapped each other by at least seven amino acids were used as antigens. As shown in Fig. 2, human MAb 1424 specifically reacted with a peptide (amino acid sequence: GGESSEELSESSFFLIT) which included amino acids 511 to 528 of NS1. This epitope may be immunodominant, as we also detected antibodies against this peptide in polyclonal rabbit sera raised against recombinant NS1 proteins (data not shown).

Both structural proteins VP1 and VP2 of parvovirus B19 are identical, except for a unique region of 226 amino acids at the amino terminus of VP1. Human MAbs 1418-1 and 1418-16 reacted specifically with the VP1 unique region which had been produced by recombinant gene technology in *E. coli*. This protein was applied as an antigen in an ELISA, as well as in Western blots. For further characterization of the antigenic epitopes recognized by human MAbs 1418-1 and 1418-16, three protein subdomains spanning the VP1 unique region were produced in *E. coli* and used as antigens (Fig. 3). The results of this analysis showed that both human MAbs recognize the same antigenic domain, located between amino acid

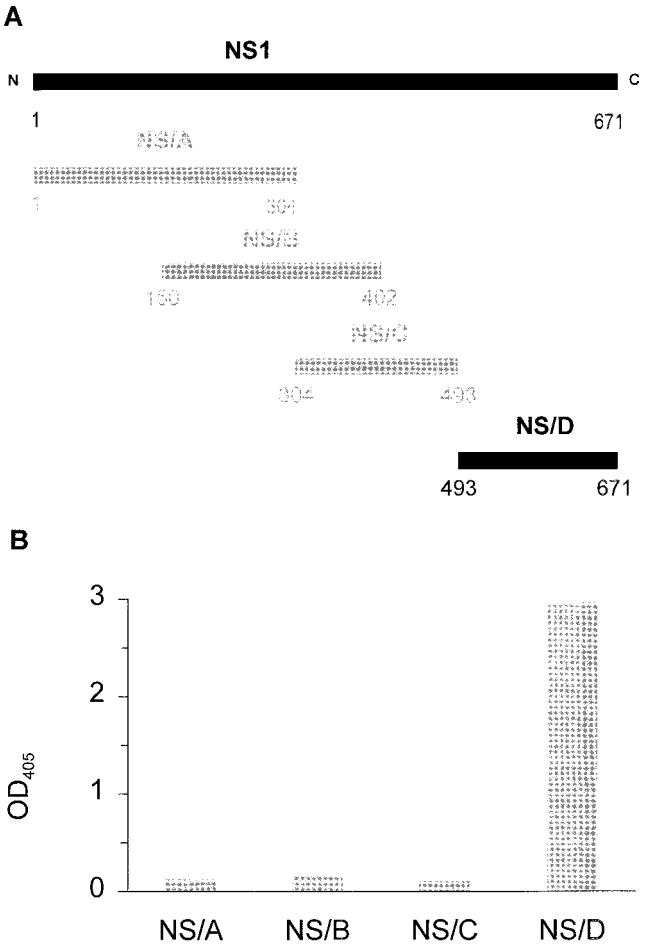

FIG. 1. Mapping of the epitope recognized by MAb 1424 with fragments derived from the NS1 protein. Fragments of the NS1 protein were produced in *E. coli*, purified, and tested in an ELISA for the ability to bind MAb 1424. (A) Schematic representation of overlapping protein fragments NS/A to NS/D. Fragments recognized by the NS1-specific MAb are in black. (B) Reactivity of MAb 1424 with different NS1 subfragments tested by ELISA. Only fragment NS/D was strongly recognized by MAb 1424. OD_{405}, optical density at 405 nm.

residues 1 and 79. Further restriction of the epitope using synthetic overlapping 20-mer peptides was not successful, suggesting that conformational parameters may contribute to the recognition of the epitope—a frequent feature of proteins interacting with particulate virus structures (20). The third human MAb, 860-55, reacted with native VP2 particles produced by recombinant baculovirus but not with denatured VP2 or VP1 proteins in ELISAs or Western blots, and further epitope mapping was not possible. Since VP2 and a major part of VP1 are identical, it has to be assumed that MAb 860-55 is also able to recognize native VP1 proteins.

Determination of B19 virus-neutralizing activities. Inhibition of parvovirus B19 infection by MAbs in human bone marrow cells was analyzed by using an erythroid-cell colony-forming assay. All four MAbs, 860-55D, 1418(1), 1418(14), and 1424, were able to markedly reduce B19 virus-induced cytotoxic effects, but to different extents (Fig. 4). MAbs 1418-1 and 1418-16, which are specific for the VP1 unique region, and MAb 860-55D, which recognizes VP2 particles, were clearly the most efficient at neutralizing parvovirus B19. In contrast, only very weak neutralizing activity was mediated by NS1-specific MAb 1424. The graphical representation of neutraliz-

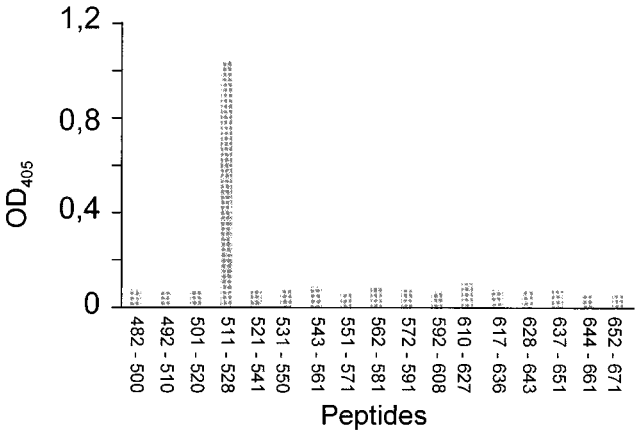

FIG. 2. Fine mapping of the epitope recognized by MAb 1424 with synthetic peptides. Shown are the reactivities of MAb 1424 with overlapping synthetic peptides covering the NS/D protein fragment in an ELISA. The numbers represent amino acid positions in the sequence of the NS1 protein. One peptide (amino acids 511 to 528) was strongly recognized by MAb 1424. OD_{405}, optical density at 405 nm.

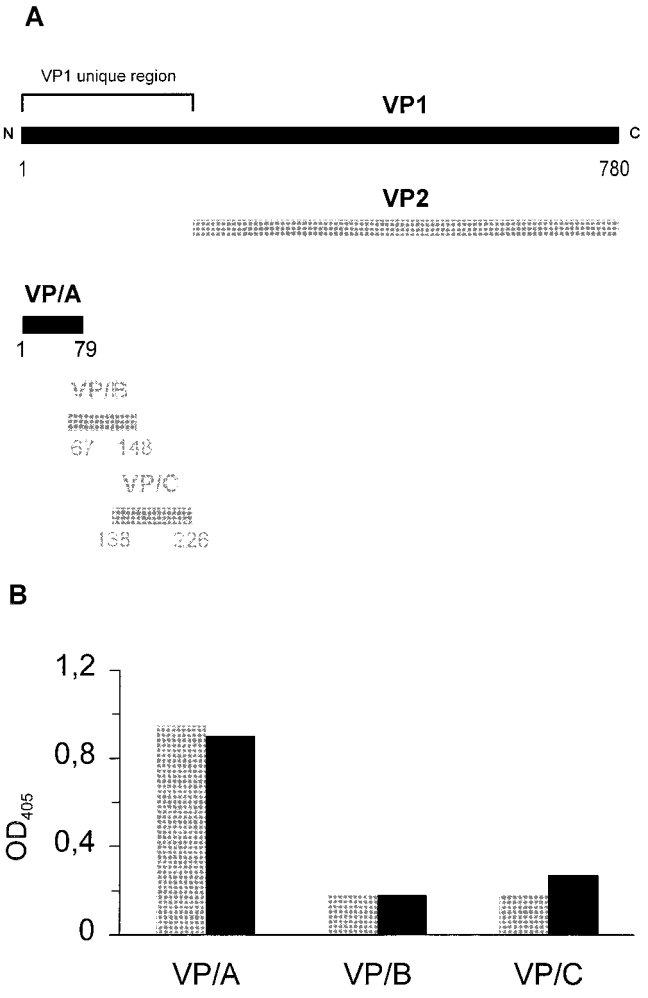

FIG. 3. Mapping of the epitope recognized by MAbs 1418-1 and 1418-16 by using VP1 protein fragments. Fragments of the VP1 protein were produced in *E. coli*, bound on ELISA plates precoated with antibodies against a polyhistidine stretch without prior purification, and tested in an ELISA for the ability to bind MAbs 1418-1 and 1418-16. (A) Schematic representation of overlapping protein fragments VP/A to VP/C. Fragments recognized by the VP1-specific MAbs are in black. (B) Reactivity of MAbs 1418-1 (grey columns) and 1418-16 (black columns) with different VP1 subfragments tested by ELISA. Only fragment VP/A was strongly recognized by both MAbs. OD_{405}, optical density at 405 nm.

ing activities shows almost identical neutralizing curves for both of the VP1-specific MAbs and a very similar pattern of reduction of virus-induced cytotoxicity for the VP2-specific MAb. For 50% neutralization, calculated mathematically by using linear interpolation, 0.08- and 0.73-μg/ml VP1- and VP2-specific human MAbs, respectively, were required; with respect to the NS1-specific MAb, a concentration of 47.7 μg/ml was necessary to achieve this level of neutralization. Control MAb 670-D, directed against gp120 of HIV-1, did not affect B19 cytotoxicity, even at the highest concentration tested. Thus, the observed inhibition of B19 infectivity mediated by MAb 1424 can be recognized as a very low-potency neutralizing activity.

Sequence determination of the variable regions of MAbs. To determine the potential identity of both VP1-specific human MAbs 1418-1 and 1418-16 and to gain insights into the conformation of IgG domains interacting with the viral antigens, the sequences of the DNA regions responsible for the synthesis of the variable domains of the heavy and light chains were determined. In addition, the families to which the respective MAbs belong were determined. The heavy chains of both VP1-specific MAbs 1418-1 and 1418-16 were classified as members of the V_H1 family, and the light chain were classified as part of the $V_\kappa3$ family (Table 2). In contrast, the heavy and light chains, respectively, of NS1-specific MAb 1424 were classified as members of the V_H3 and $V_\lambda1$ families.

Comparison of the cDNA sequences encoding the variable domains of MAbs 1418-1 and 1418-16 revealed identity except for four base pairs, resulting in only one amino acid change in the light chain (lysine to arginine in the third complementarity-determining region, CDR3). Since both sequences are derived from antibodies produced by the same healthy individual but secreted from separate Epstein-Barr virus-transformed B-cell clones, the sequence differences may have resulted from in vivo somatic mutations in the light-chain variable regions. Sequence analysis of MAb 1424 showed major differences, particularly in the variable domains of the antibodies.

DISCUSSION

The seroprevalence of IgG antibodies against structural proteins VP1 and VP2 of human parvovirus B19 is 40 to 70% among adults, depending on the method of antibody detection and the average age of the population examined (11, 25). The frequency of NS1-specific IgG is low (ca. 15%) but may be elevated in persons with persistent B19 virus infections or those with immunological inability to eliminate the virus (32, 33). The results presented here show that detectable levels of circulating B cells secreting B19 virus-specific antibodies are not restricted to either acutely or persistently infected persons but are also found in healthy individuals. This may indicate that either the immune system is repeatedly stimulated by B19 virus reexposure or cross-specific antigens or parvovirus B19 may persist at low concentrations in immunocompetent individuals at locations and in cells where it cannot easily be detected by nested PCR. The second hypothesis is supported by observations recently published by Söderlund and coworkers showing B19 virus-specific DNA sequences in synovial cells of 43% of otherwise healthy trauma patients (28) and by examinations done by Cassinotti and coworkers, who detected

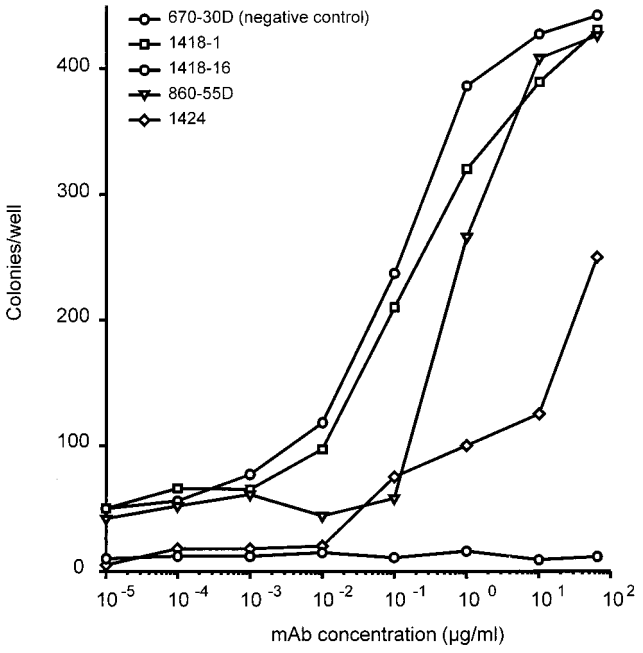

FIG. 4. Capacities of human MAbs to protect erythroid progenitor cells from the cytotoxic effects of infectious parvovirus B19. Neutralization was studied in the CFU-E-derived colony inhibition assay. The results of one of four consistent experiments are illustrated. Means of duplicate determinations of erythroid-cell colonies are shown. In this experiment, the average number of colonies formed in the absence of virus and antibody was 450 per well.

B19 viral DNA in the bone marrow of asymptomatic individuals (6).

At the time this study was started, a total of 12 MAbs with B19 virus-neutralizing activities had been described, but only 2 of those are able to recognize linear epitopes, probably located at the surface of the virus capsid: 1 mouse MAb had been mapped to amino acids 284 to 304 of the VP1/VP2 protein (34), and 1 other had been mapped to amino acids 555 to 571 of the same polypeptide (24). Both epitopes are not part of the VP1 unique region. Thus, human MAbs 1418-1 and 1418-16 are the first B19 virus-neutralizing MAbs the epitopes of which are located in the VP1 unique region. Based on experiments with polyclonal rabbit sera, it was suggested by Saikawa and coworkers that several linear neutralizing epitopes are located in the regions between amino acids 46 and 93 and 148 and 295 of the VP1 protein (23). Also, by using polyclonal rabbit sera, they defined a B19 virus-neutralizing epitope within amino acids 31 to 51 of the VP1 unique region. Based on these and other data obtained by using B19 virus-specific antibodies generated in animal systems, most of the neutralizing epitopes present in the VP1 unique region appeared to be linear, in contrast to those located in major capsid protein VP2. Also, VP2-specific human MAb 860-55D recognizes a conformational epitope defined by the structure of VP2 proteins in the B19 virus particle. The epitopes recognized by VP1-specific human MAbs 1418-1 and 1418-16, however, could not be mapped by using synthetic peptides. Nevertheless, both antibodies reacted with the denatured VP1 protein on Western blots. To exclude the possibility that the peptides were incorrect, we verified amino acid sequences by mass spectrometry and used them in an ELISA to analyze the reactivities of VP1-specific polyclonal rabbit sera (data not shown). These investigations were consistent with the originally determined sequences. Therefore, it may be concluded that protein conformation, which cannot be represented by small peptides, may contribute to the nature of the epitope and to antibody binding. By using subfragments of the VP1 protein produced in *E. coli* as antigens in an ELISA, specific reactions could be correlated with the region of the protein spanning amino acids 1 to 79. Since a positive reaction could not be shown with amino acids 67 to 148 of the VP1 protein, the antigenic domain is assumed to be located at amino-terminal residues 1 to 66.

The linear epitopes located in the VP1 unique region and the immune reaction raised against these domains appear to be important for elimination of B19 viremia. Patient sera with low reactivities against those epitopes on immunoblots also displayed poor B19 virus-neutralizing activities (17). Since human MAbs 1418-1 and 1418-16 show strong reactivities in Western blots, it may be concluded that their corresponding epitopes represent potential candidates for the production of a vaccine based on recombinant VP1 subfragments for the prevention of human parvovirus B19 infections.

Human MAbs may be useful as therapeutic agents for treatment of B19 virus infections. Patients with chronic B19 virus infections and anemia have been successfully treated with commercially available immunoglobulin preparations containing high titers of B19 virus-specific antibodies (10, 16). Human MAbs 1418-1, 1418-16, and 860-55, directed against the B19 virus capsid proteins, have been characterized as part of subclass IgG1. IgG1 antibodies have a long half-life of about 3 weeks and are very effective in mediating phagocytosis by mononuclear cells. Since these human MAbs are directed against naturally occurring infectious B19 virus particles and are able to neutralize the virus even at low concentrations, they may be ideal candidates for passive immunization. Furthermore, the protein domain recognized by these antibodies is genetically stable: the amino acid sequences obtained from eight virus isolates collected at different locations and in different years displayed a low degree of variability of 3.5% in that part of the VP1 unique region (data not shown). In addition, problems generally associated with the use of immunoglobulin preparations derived from pooled human sera, such as contamination with other infectious agents, are avoided.

The epitope recognized by NS1-specific human MAb 1424 is located in the carboxy terminus of the protein, between amino acids 511 and 528, and has a linear structure. Furthermore, this epitope appears to be immunodominant, as we could also show the presence of antibodies against peptides spanning this domain in polyclonal rabbit sera raised against recombinant NS1 proteins (data not shown). The function of this portion of the multifunctional NS1 protein has not yet been analyzed. The carboxy-terminal region of the largest nonstructural protein of simian parvovirus (4) and minute virus of mice (18) is known to be involved in promoter transactivation. Thus, human MAb 1424 might be a useful tool for studying parvovirus genetic regulation in vitro.

Our observation that the NS1-specific human MAb is weakly but significantly active in B19 virus neutralization is surprising, since the NS1 protein has not been described as part of infectious B19 virus particles (8). In contrast, the NS1 protein of minute virus of mice remains associated with the ends of the genome and is transiently located on the exterior of the virion, where it is accessible to both antibodies and enzymes (9). It cannot be excluded that the NS1 protein of parvovirus B19 may be partly or temporarily associated with infectious particles at very low concentrations. In this case, NS1-specific antibodies may bind to the virus surface and either block virus adsorption to the globoside receptor by steric hindrance or influence the

function of the NS1 protein as part of the infectious particle after virus uptake.

This finding, however, may explain the significance of NS1-specific antibodies in some patients (32, 33). These antibodies are probably not an irrelevant by-product of the immune reaction induced by release of the NS1 protein due to virus-induced cell lysis but contribute to elimination of the virus and to the pathogenesis of B19 virus infections.

ACKNOWLEDGMENTS

This work was supported by Deutsche Forschungsgemeinschaft grant Mo620/5-1 and by the Department of Veterans Affairs. Andreas Gigler was supported by a grant from the University of Regensburg (Förderung des wissenschaftlichen Nachwuchses), and Andrea Hemauer was supported by the Studienstiftung des Deutschen Volkes.

We thank Ulla Raab for competent and helpful discussion of the experiments and Astrid Brunner and Karin Beckenlehner for technical assistance. We thank F. Dunn (Med Immune) for preparation of recombinant VP2 protein and M. Motz (Mikrogen GmbH, Munich, Federal Republic of Germany) for recombinant VP1 and NS1 proteins. We also acknowledge the help of healthy volunteers from whom the materials were derived.

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