JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2002, p. 933–936 Vol. 40, No. 3
0095-1137/02/$04.00+0 DOI: 10.1128/JCM.40.3.933–936.2002

Prevalence of Parvovirus B19 and Parvovirus V9 DNA and Antibodies in Paired Bone Marrow and Serum Samples from Healthy Individuals

Erik D. Heegaard,[1,2]* Bodil Laub Petersen,[3] Carsten J. Heilmann,[4] and Allan Hornsleth[1]

Departments of Clinical Microbiology,[1] Pathology,[3] and Pediatrics, Clinic II,[4] University State Hospital, Rigshospitalet, and Immunology, University of Copenhagen,[2] Copenhagen, Denmark

Received 25 April 2001/Returned for modification 8 October 2001/Accepted 15 December 2001

Parvovirus B19 (hereafter referred to as B19) exhibits a marked tropism to human bone marrow (BM), and infection may lead to erythema infectiosum, arthropathy, hydrops fetalis, and various hematologic disorders. Recently, a distinct parvovirus isolate termed V9 with an unknown clinical spectrum was discovered. In contrast to the many studies of B19 serology and viremia, valid information on the frequency of B19 or V9 DNA in the BM of healthy individuals is limited. To develop a reference value, paired BM and serum samples from healthy subjects were tested for the presence of B19 and V9 DNA and specific antibodies. Immunoglobulin M (IgM) was not found in any of the serum samples. The prevalence of IgG showed a gradual and steady increase from 37% in children aged 1 to 5 years to 87% in people aged >50 years. When 190 well-characterized subjects were examined, B19 DNA was detected in the BM of 4 individuals (2.1%; 95% confidence interval, 0.58 to 5.3%) while none of the paired serum samples showed evidence of circulating viral DNA. V9 DNA was not found in any of the BM or serum samples. The finding of B19 DNA probably indicated a primary infection in one 7-year-old individual and reinfection or reactivation of persistent infection in the remaining three persons, aged 47 to 58 years. Serving as a benchmark for future studies, these findings are useful when interpreting epidemiologic data, performing BM transplantation, or considering clinical implications of parvovirus infection.

Human parvovirus B19 (hereafter referred to as B19) is a member of the genus *Erythrovirus* of the family *Parvoviridae* (18). B19 exhibits a marked tropism to human bone marrow (BM) and replicates only in erythroid progenitor cells (3). Infection may lead to erythema infectiosum, arthropathy, hydrops fetalis, and various hematologic disorders, including aplastic crisis, chronic anemia, and idiopathic thrombocytopenic purpura (3). Diagnosis of B19 relies on serology and the detection of viral DNA by PCR or dot blot analysis. A possible emerging parvovirus isolate termed V9 with an unknown clinical spectrum and markedly different from B19 (>11% nucleotide disparity) was recently discovered (19). Sequencing, combined with PCR studies, has since demonstrated the need for specific and differentiated techniques when examining samples for possible B19 or V9 viremia (11). Conversely, cloning and production of the V9 capsid proteins and subsequent enzyme-linked immunosorbent assay studies have revealed a 100% serologic cross-reactivity between the B19 and V9 isolates (13).

The prevalence of immunoglobulin G (IgG) antibodies directed against B19 ranges from 15 to 60% in children 6 to 19 years old: and from 30 to 60% in adults and is more than 85% in the geriatric population (1, 7, 22). The frequency of B19 viremia in voluntary blood donors has been estimated at rates of 1:167 to 1:35,000 (11, 14, 17, 22–24). As opposed to the many studies on B19 serology and viremia, information on the presence of B19 DNA in the BM of healthy individuals is limited. Though no serum was available for comparison, B19 DNA was discovered in 4 of 45 (9%) healthy bone donors (5), while another study reported no evidence of B19 DNA in the BM of 13 BM donors (9). When paired serum and BM samples have been examined for B19 DNA, it has been shown that serum samples are only positive in a fraction (0 to 50%) of patients with positive BM samples, which may be explained by the tropism of B19 (2, 5, 6, 15, 16). Likewise, monitoring the clearance of virus by detection of DNA in sequential samples is also less sensitive when serum samples are used rather than BM. Moreover, no study of the prevalence of V9 in the BM of patients or in the general population exists.

To develop a reference value, we examined paired BM and serum samples from healthy subjects for the presence of B19 and V9 DNA and specific antibodies. This material may be used as a point of reference for future studies of parvovirus infection and when discussing possible persistent infection or reinfection by this pathogen.

MATERIALS AND METHODS

Materials. Paired serum and BM samples from 153 consecutive healthy Danish BM donors for transplantation, assembled from 1981 to 1989 ($n = 24$) and 1995 to 2000 ($n = 129$), were retrieved (Table 1). The median age was 28 years, with 38 samples available from individuals in the chief B19-susceptible age (1 to 20 years). The panel of samples was supplemented by BM samples (no sera were available) from 37 infants with sudden infant death syndrome (SIDS) obtained from 1988 to 1995, which were believed to be unbiased in regard to parvovirus infection.

PCR procedure. All BM samples were initially tested by PCR for the presence of part of the human β-globin gene, ensuring the presence of intact DNA material for subsequent parvovirus detection (10, 20). Serum and BM samples were then examined by nested PCR, which involved consensus primers for the primary rounds of amplification followed by separate primers for the amplification of B19 and V9 DNA, respectively (11). The nested assay had a consistent sensitivity and was able to correctly identify 1 to 10 copies of target DNA in a 5-μl sample volume (200 to 2,000 copies/ml) (11).

* Corresponding author. Mailing address: IMMI, Panum Institute, Bldg. 24.2, Blegdamsvej 3, 2200 Copenhagen N, Denmark. Phone: 45 35 32 76 79. Fax: 45 35 32 78 53. E-mail: e.heegaard@immi.ku.dk.

TABLE 1. Age and gender characteristics of 190 individuals enrolled in the study

Age (yr)	No. (%) of females	No. (%) of males	Age (yr) (median/mean)	Total no. of subjects
0–1	15 (41)	22 (59)	0.25/0.3	37
1–5	4 (50)	4 (50)	3.5/3.3	8
6–10	5 (56)	4 (44)	8.7/8.6	9
11–20	13 (62)	8 (38)	16.5/16.6	21
21–50	43 (47)	48 (53)	36.4/36.2	91
>50	9 (38)	15 (62)	55.1/56.8	24
Total	89 (47)	101 (53)	28/27[a] 34.9/33.4[b]	190

[a] Including all age groups.
[b] Excluding infants aged 0 to 1 year.

Sequencing reactions. To rule out the possibility of contamination and to characterize the viral strains, sequencing of a 390-bp fragment of the VP2 gene (nucleotides 4071 to 4460) (21) in all positive samples and the corresponding positive controls was performed as previously described (11).

Serologic tests. The presence of specific B19 and V9 IgM and IgG antibodies was determined using V9 recombinant VP2 capsid proteins in a newly developed enzyme-linked immunosorbent assay, which shows a 100% serologic cross-reactivity between the B19 and V9 isolates (13).

Morphology. Positive BM samples were reviewed histologically by one of the authors (B.L.P.); morphologic features, including the presence of megakaryocytes and giant pronormoblasts, were noted, and myeloid/erythroid ratios were calculated.

Ethical considerations. The study was approved by The Scientific Ethics Committee of Copenhagen, Denmark (reference 01-360/94).

RESULTS

PCR procedure. All positive controls were positive, and all negative controls remained negative during the PCR experiments. The human β-globin gene was detected in every BM sample, allowing all of the initially collected samples to be included in the study. B19 DNA was detected in four BM samples (2.1%; 95% confidence interval, 0.58 to 5.3%, or 2.6% in individuals >1 year old) (subjects 1 to 4), while none of the paired serum samples showed evidence of circulating viral DNA (Table 2). V9 DNA was not found in any of the BM or serum samples.

Sequencing reactions. When sequenced and aligned to the reference B19 isolate *pvbaua* (21), each of the four B19-positive samples showed minor and individual differences in the nucleotide sequence compared to the B19 positive control (Table 3).

Serologic tests. B19 IgM was not found in any of the serum samples (Table 2). The prevalence of B19 IgG showed a gradual and steady increase from 37% in children aged 1 to 5 years to 87% in people aged >50 years, and the presence of B19 IgG was significantly correlated with higher age when compared to seronegative individuals ($P = 0.009$; Mann-Whitney rank sum test). All of the B19 DNA-positive individuals exhibited specific IgG.

Morphology. The cellularity of the specimens and the morphology of megakaryocytes were normal. The myeloid/erythroid ratio ranged from 3:1 to 5:1, and the erythropoietic cells were normal. Giant pronormoblasts were not noticed, and immunohistochemistry for parvovirus was negative in all cases.

Characterization of positive subjects. The ages of the B19-positive individuals were 7, 47, 56, and 58 years, respectively, and none of them had received blood products. Prior to BM transplantation, all donors were subjected to a physical examination and a variety of serologic and morphologic tests. All persons involved, including the four B19-positive donors, were deemed healthy without signs of active or recent illness.

DISCUSSION

B19 is a ubiquitous infectious agent and is transmitted by the respiratory route or by blood and various blood products (3). Numerous studies of the occurrence of viral DNA and specific antibodies in the general population have been described (1, 7, 11, 14, 17, 22–24). Simultaneous examination of BM and serum samples from patients for the presence of B19 DNA has re-

TABLE 2. Detection of parvovirus antibodies and specific DNA in blood and BM in 190 individuals enrolled in the study

Age (yr)	No. (%) of subjects with[a]:							
	Antibodies				Serum		BM	
	IgM^- IgG^-	IgM^+ IgG^-	IgM^+ IgG^+	IgM^- IgG^+	B19 DNA	V9 DNA	B19 DNA	V9 DNA
0–1	NA	NA	NA	NA	NA	NA	0	0
1–5	5 (63)	0	0	3 (37)	0	0	1	0
6–10	5 (56)	0	0	4 (44)	0	0	0	0
11–20	8 (38)	0	0	13 (62)	0	0	0	0
21–50	20 (22)	0	0	71 (78)	0	0	1	0
>50	3 (13)	0	0	21 (87)	0	0	2	0
Total	41 (27)	0	0	112 (73)	0	0	4	0

[a] NA, sample not available.

TABLE 3. Sequence alignment of a 390-bp fragment of the B19 VP2 capsid gene (nucleotides 4071 to 4460) from four B19-positive clinical isolates and the positive control compared to the princeps isolate *pvbaua*

Viral isolate	Nucleotide changes		Amino acids	
	No. (%)	Position[a]	Codons	Change[b] (%)
Subject 1	1 (0.25)	4300	TAC→TAT	None (0)
Subject 2	4 (1)	4171	ATA→ATG	Ile→Met (1.5)
		4192	CAG→CAA	None
		4220	GAG→AAG	Glu→Lys
		4400	CTG→TTG	None
Subject 3	2 (0.5)	4182	TCT→TTT	Ser→Phe (0.8)
		4300	TAC→TAT	None
Subject 4	3 (0.8)	4162	GTC→GTA	None (1.5)
		4182	TCT→TTT	Ser→Phe
		4264	CAG→CAT	Gln→His
Control	7 (1.8)	4160	GTC→ATC	Val→Ile (3.1)
		4178	ATT→GTT	Ile→Val
		4205	AAC→GAA	Asn→Glu
		4207	AAC→GAA	Asn→Glu
		4267	CTA→CTG	None
		4300	TAC→TAT	None
		4321	CAG→CAA	None

[a] Nucleotide position refers to the position according to the sequence published by Shade et al. (21).
[b] Changes in 130 amino acids derived from the 390-bp fragment.

vealed discrepancies, with B19 being briefly or continuously detected in BM following clearance of virus in the serum. The tropism and replication of B19 in BM and subsequent discharge of virus into the circulation may explain the discordant results. Thus, detection of viral DNA in serum probably at best merely mirrors a current infection taking place in the BM. Accordingly, in low-grade or chronic infections, the viral load in serum may be diminished to a degree below the threshold of detection, while BM samples remain positive. No major systematic data exist on the prevalence of B19 or V9 in the BM of healthy subjects, let alone paired serum and BM samples. To generate a reference value, the prevalences of both B19 and V9 DNA and antibodies were investigated using paired serum and BM samples from healthy subjects.

BM samples from 190 individuals were retrieved, and apart from 37 children with SIDS, serum was available in all cases. The nucleotide discrepancies detected by sequencing indicate that the B19-positive samples represent truly positive samples. B19 has been known to undergo little genetic variation (1 to 2% of the genome) (8), and we found comparable genetic variations in the B19-positive samples.

B19 DNA was not detected in any of the 153 serum samples (Table 2), reflecting the fact that B19 viremia is an uncommon occurrence among blood donors. Likewise, the serologic results support previous findings in which the prevalence of IgG shows a gradual rise throughout life. Accordingly, in children aged 1 to 5 years approximately one in three exhibited B19 IgG, while older children and adults had an even higher prevalence of 44 to 87% (Table 2).

The prevalence of B19 and V9 DNA in the BM of the general population has hitherto been unresolved. Initial findings in two studies involving only 45 (5) and 13 (9) people, with no serum samples or clinical data available for comparison, showed discrepant results, with the prevalence of B19 DNA in BM estimated at 0 and 9%, respectively. Presently, when 190 well-characterized subjects were examined, B19 DNA was detected in the BM of 4 individuals (2.1%), indicating that a baseline incidence of B19 DNA of a few percent in the BM of the general population is to be expected. Given the discrepant findings when paired serum and BM samples were examined, as well as in the results from previous reports (2, 5, 6, 15, 16), it is likely, despite the advent of the highly sensitive PCR technique, that a higher level of viral replication is necessary for detection of B19 DNA in serum as opposed to BM.

The presence of viral DNA or specific IgM is generally accepted as an indication of an ongoing or recent primary infection. Thus, detection of B19 DNA in subject 1 probably represented the tail end of a primary infection, given the age (7 years) and the fact that no IgM was present. However, considering the ages of the remaining three positive individuals (47 to 58 years), the finding of viral DNA may also represent reactivation of persistent infection or reinfection. Consequently, a mechanism analogous to that of varicella might be possible, in which an age-related diminished level of antibodies or cell-mediated immunity can lead to low-grade reactivation or reinfection, with detectable levels of B19 DNA possibly found exclusively in the BM without accompanying overt clinical symptoms. It has been proposed that B19 screening of blood components destined for children with malignancies be implemented (4), and this may well include testing of BM donors prior to BM transplantation, given the present results and the discovery of symptomatic B19 infection transmitted by BM (12).

In conclusion, B19 DNA may be detected in BM, but not serum, in a few percent of healthy individuals. Serving as a benchmark for future studies, these findings are of importance when interpreting epidemiologic data or considering the clinical implications of parvovirus infection.

ACKNOWLEDGMENTS

We are grateful to B. F. Vestergaard, Virology Department, State Serum Institute, who collected stored clinical serum samples from BM donors, and H. P. Hougen, Forensic Department, Rigshospitalet, for generously sharing BM material from SIDS children.

REFERENCES

1. **Anderson, L. J.** 1987. Role of parvovirus B19 in human disease. Pediatr. Infect. Dis. J. **6:**711–718.
2. **Broliden, K., T. Tolfvenstam, S. Ohlsson, and J. I. Henter.** 1998. Persistent B19 parvovirus infection in pediatric malignancies. Med. Pediatr. Oncol. **31:**66–72.
3. **Brown, K. E.** 2000. Haematological consequences of parvovirus B19 infection. Baillieres Best Pract. Res. Clin. Haematol. **13:**245–259.
4. **Brown, K. E., N. S. Young, B. M. Alving, and L. H. Barbosa.** 2001. Parvovirus B19: implications for transfusion medicine. Summary of a workshop. Transfusion **41:**130–135.
5. **Cassinotti, P., G. Burtonboy, M. Fopp, and G. Siegl.** 1997. Evidence for persistence of human parvovirus B19 DNA in bone marrow. J. Med. Virol. **53:**229–232.
6. **Cassinotti, P., G. Siegl, B. A. Michel, and P. Bruhlmann.** 1998. Presence and significance of human parvovirus B19 DNA in synovial membranes and bone marrow from patients with arthritis of unknown origin. J. Med. Virol. **56:** 199–204.
7. **Cohen, B. J., and M. M. Buckley.** 1988. The prevalence of antibody to human parvovirus B19 in England and Wales. J. Med. Microbiol. **25:**151–153.
8. **Erdman, D. D., E. L. Durigon, Q. Y. Wang, and L. J. Anderson.** 1996. Genetic diversity of human parvovirus B19: sequence analysis of the VP1/VP2 gene from multiple isolates. J. Gen. Virol. **77:**2767–2774.

9. **Foto, F., K. G. Saag, L. L. Scharosch, E. J. Howard, and S. J. Naides.** 1993. Parvovirus B19-specific DNA in bone marrow from B19 arthropathy patients: evidence for B19 virus persistence. J. Infect. Dis. **167:**744–748.
10. **Heegaard, E. D., H. Hasle, N. Clausen, A. Hornsleth, and G. B. Kerndrup.** 1996. Parvovirus B19 infection and Diamond-Blackfan anaemia. Acta Paediatr. **85:**299–302.
11. **Heegaard, E. D., I. P. Jensen, and J. Christensen.** 2000. Novel PCR assay for differential detection and screening of erythrovirus B19 and erythrovirus V9. J. Med. Virol. **65:** 362–367.
12. **Heegaard, E. D., and P. B. Laub.** 2000. Parvovirus B19 transmitted by bone marrow. Br. J. Haematol. **111:**659–661.
13. **Heegaard, E. D., K. Qvortrup, and J. Christensen.** 2002. Baculovirus expression of erythrovirus V9 capsids and screening by ELISA: serologic cross-reactivity with erythrovirus B19. J. Med. Virol. **66:**246–252.
14. **Jordan, J., B. Tiangco, J. Kiss, and W. Koch.** 1998. Human parvovirus B19: prevalence of viral DNA in volunteer blood donors and clinical outcomes of transfusion recipients. Vox Sang. **75:**97–102.
15. **Lundqvist, A., T. Tolfvenstam, J. Bostic, M. Soderlund, and K. Broliden.** 1999. Clinical and laboratory findings in immunocompetent patients with persistent parvovirus B19 DNA in bone marrow. Scand. J. Infect. Dis. **31:** 11–16.
16. **Lundqvist, A., T. Tolfvenstam, M. Brytting, C. M. Stolt, K. Hedman, and K. Broliden.** 1999. Prevalence of parvovirus B19 DNA in bone marrow of patients with haematological disorders. Scand. J. Infect. Dis. **31:**119–122.
17. **McOmish, F., P. L. Yap, A. Jordan, H. Hart, B. J. Cohen, and P. Simmonds.** 1993. Detection of parvovirus B19 in donated blood: a model system for screening by polymerase chain reaction. J. Clin. Microbiol. **31:**323–328.
18. **Murphy, F. A., C. M. Fauquet, D. H. L. Bishop, S. A. Ghabrial, A. W. Jarvis, G. P. Martelli, M. A. Mayo, and M. D. Summers.** 1995. Virus taxonomy: classification and nomenclature of viruses. Sixth report of the International Committee on Taxonomy of Viruses. Springer-Verlag, New York, N.Y.
19. **Nguyen, Q. T., C. Sifer, V. Schneider, X. Allaume, A. Servant, F. Bernaudin, V. Auguste, and A. Garbarg-Chenon.** 1999. Novel human erythrovirus associated with transient aplastic anemia. J. Clin. Microbiol. **37:**2483–2487.
20. **Saiki, R. K., S. Scharf, F. Faloona, K. B. Mullis, G. T. Horn, H. A. Erlich, and N. Arnheim.** 1985. Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science **230:**1350–1354.
21. **Shade, R. O., M. C. Blundell, S. F. 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.
22. **Tsujimura, M., K. Matsushita, H. Shiraki, H. Sato, K. Okochi, and Y. Maeda.** 1995. Human parvovirus B19 infection in blood donors. Vox Sang. **69:**206–212.
23. **Wakamatsu, C., F. Takakura, E. Kojima, Y. Kiriyama, N. Goto, K. Matsumoto, M. Oyama, H. Sato, K. Okochi, and Y. Maeda.** 1999. Screening of blood donors for human parvovirus B19 and characterization of the results. Vox Sang. **76:**14–21.
24. **Yoto, Y., T. Kudoh, K. Haseyama, N. Suzuki, T. Oda, T. Katoh, T. Takahashi, S. Sekiguchi, and S. Chiba.** 1995. Incidence of human parvovirus B19 DNA detection in blood donors. Br. J. Haematol. **91:**1017–1018.