NOTES

Parvovirus among Patients with Cytopenia of Unknown Origin in Brazil: a Case-Control Study[▽]

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The molecular prevalence of human parvovirus B19V (B19V) in bone marrow (BM) samples from 120 cases with cytopenias of unknown etiology was compared with that in samples from 45 BM donors (control group 1) and 120 oncohematological patients (control group 2) to determine the role that B19V genotypes may play in unexplained cytopenias. Of the 285 participants, the BM samples of 39 (13.7%) contained B19V DNA (21 with genotype 1, 5 with genotype 2, and 13 with genotype 3). The prevalences of B19V were similar between case and control subjects (15.0% versus 12.7%, respectively). Genotypes 2 and 3 were associated with older age and were detected in similar proportions between case and control group 2 subjects. The results of this study do not support a role for B19V genotype variants in the etiology of unexplained cytopenias.

Human parvovirus B19 (B19V), which belongs to the genus *Erythrovirus* of the family *Parvoviridae*, is a tiny single-stranded DNA virus that propagates in actively dividing erythroid lineage progenitors of bone marrow (BM) and blood, inhibiting erythropoiesis (1). Comparisons of B19V genomic sequences from different isolates identified 3 genetically distinct B19V genotypes with more than 10% genetic variability among them. These were designated genotype 1 (B19-related viruses), genotype 2 (A6-related viruses), and genotype 3 (V9-related viruses) (5). Infection with B19V genotype 1 has been associated with a wide range of human diseases, including erythema infectiosum, arthropathy, transient aplastic crisis, persistent anemia, and hydrops fetalis (6).

We have previously shown that genotype 3 accounts for approximately 50% of the parvovirus-positive samples in our center (4); however, the clinical significance of this finding has not yet been determined. In this study, we aimed to determine if the presence of B19V genotypes 2 and 3 isolated from BM cells is associated with cytopenias of unknown etiology.

An unmatched 1:2 case-control study was conducted from September 2006 to May 2009. Based on the results of our previous study and on the following assumptions ($\alpha = 0.05$; power = 0 to 80; prevalence rate of B19V in patients = 17.5%; odds ratio for B19V infection = 2.8; ratio of controls to cases = 2:1), it was determined that a sample size of 122 cases was needed to demonstrate a link between B19V infection and

cytopenia. Accordingly, for this study, we recruited a total of 120 cases fulfilling the recommended diagnostic criteria for idiopathic cytopenia. These criteria define idiopathic cytopenia as cytopenia in one or more cell lineages (i) that persists for at least 6 months and (ii) that cannot be explained by any other hematological or nonhematological disease. Two control groups were chosen to enhance the power of the study. Control group 1 consisted of 45 BM donors, and group 2 included 120 patients with oncohematological disorders (Table 1). We were unable to identify two controls for every case, and the total number of controls was thus limited to 165.

Peripheral blood (PB) (5 ml) and BM samples were collected from all participants. B19V DNA was extracted and amplified as described previously (4). PCR products were purified and subsequently sequenced in both directions using the internal PCR primers. The resultant sequences were aligned with B19V GenBank sequences that showed high-scoring matches with our sequences using ClustalX and then analyzed phylogenetically as previously described (4). The plasma (PL) samples were tested for the presence of B19V IgM and IgG using the Biotrin parvovirus B19 IgM and IgG enzyme immunoassay kits (Biotrin International Ltd., Dublin, Ireland).

Of the 285 individuals evaluated, 152 (53%) were male and 133 (47%) were female. BM donors were younger (median age = 35 years) than both case patients (median age = 55 years) and control group 2 (median age = 60 years; $P < 0.01$). The rates of anemia (hemoglobin less than 11 g/dl), thrombocytopenia (platelet count below 150,000/ μ l), and leukopenia (total leukocyte count, $<4,000$ cells/ mm^3) in the cases were 64%, 54%, and 17%, respectively. In control group 2, the rates were 39%, 41%, and 2%, respectively.

As shown in Table 1, the B19V partial genome was detected

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TABLE 1. Demographic characteristics of and parvovirus presence in the cases and controls according to genotype

Characteristic	No. (%) of subjects positive for parvovirus			Total
	Overall PCR	Genotype 1	Genotypes 2 and 3	
Group				
Cases	18 (15.0)	11 (9.2)	7 (5.8)	120
Control 1	6 (13.3)	5 (11.1)	1 (2.2)	45
Control 2	15 (12.5)	5 (4.2)	10 (8.3)	120
Gender				
Male	20 (13.1)	11 (7.2)	9 (5.9)	152
Female	20 (15.0)	11 (8.2)	9 (6.7)	133
Median age in yrs	55.5	47	62.5 ^a	

^a Difference between genotypes 2/3 and genotype 1, $P < 0.01$ (Mann-Whitney test).

in 39 (13.7%) of 285 BM samples and 11 (27.5%) of these had detectable virus in their peripheral blood. Of these 39 participants, 21 (53.9%) were infected with genotype 1, 5 (12.8%) with genotype 2, and 13 (33.3%) with genotype 3. The overall prevalences of B19V were similar between cases and controls (15.0% versus 12.7%, respectively; odds ratio [OR], 1.2; 95% confidence interval [CI], 0.58 to 2.51, $P = 0.5$). In the 39 medical reports of participants with detectable B19V DNA, there were 3 cases and 1 neoplastic patient from control group 2 who had blood transfusions before their BM samples were collected. The prevalences of genotypes 2 and 3 were also similar between the cases (5.8%) and control group 2 (6.7%; $P = 0.7$). Genotype 3 was detected only in one specimen from the BM donor control group by PCR analysis. The prevalences of circulating B19V were not significantly different between cases (5.0%) and controls (4.2%; $P = 0.8$).

Based on the serological results shown in Table 2, three groups were identified: (i) one group of 3 cases with acute or recent B19V infection (positive test for IgM B19V antibodies

TABLE 2. Description of the PCR-positive cases^a

Group	Sequence ID	Age (yr)	Sample(s) PCR positive	Genotype	Serology (ELISA) result	Clinical history
Cases	07BR EC01	36	PB and BM	1	IgM and IgG	Pure red cell aplasia, HIV, hepatitis
	07BR EC02	61	BM	1	IgG	Pancytopenia
	07BR EC03	27	BM	1	IgG	Anemia, skin rash, arthralgia
	07BR EC04	84	BM	3	IgG	Pure red cell aplasia
	07BR EC05	54	PB and BM	3	IgM and IgG	Anemia, skin rash, arthralgia
	07BR EC06	53	PB and BM	1	IgM and IgG	Pure red cell aplasia, skin rash, arthralgia
	07BR EC07	76	BM	3	Negative	Anemia, skin rash, arthralgia
	07BR EC08	33	BM	1	IgG	Leukopenia
	07BR EC09	76	BM	1	IgG	Thrombocytopenia
	07BR EC10	72	BM	2	IgG	Thrombocytopenia
	07BR EC11	56	BM	1	IgG	Anemia, arthralgia
	07BR EC12	43	BM	1	IgG	Anemia and thrombocytopenia
	07BR EC13	87	PB and BM	1	Negative	Pancytopenia
	07BR EC14	55	PB and BM	3	IgG	Thrombocytopenia
	07BR EC15	61	PB and BM	1	IgG	Anemia, skin rash, hepatitis
	07BR EC16	69	BM	2	IgG	Thrombocytopenia, skin rash, arthralgia, hepatitis
	07BR EC17	62	BM	3	IgG	Thrombocytopenia, arthralgia
	07BR EC18	53	BM	1	IgG	Leukopenia, arthralgia
Group 1	07BR EGP2_01	31	BM	1	IgG	Asymptomatic
	07BR EGP2_02	30	BM	1	IgG	Asymptomatic
	07BR EGP2_03	31	BM	1	IgG	Asymptomatic
	07BR EGP2_04	33	BM	3	IgG	Asymptomatic
	07BR EGP2_05	51	BM	1	Negative	Asymptomatic
	07BR EGP2_06	45	BM	1	IgG	Asymptomatic
Group 2	07BR EGP3_01	63	PB and BM	3	IgG	Non-Hodgkin lymphoma
	07BR EGP3_02	74	BM	3	IgG	Chronic lymphocytic leukemia
	07BR EGP3_03	57	BM	1	IgG	Hodgkin lymphoma
	07BR EGP3_04	57	BM	1	Negative	Hairy cell leukemia
	07BR EGP3_05	73	PB and BM	2	Negative	Chronic lymphocytic leukemia
	07BR EGP3_06	47	PB and BM	3	IgG	Chronic myelogenous leukemia
	07BR EGP3_07	63	BM	3	IgG	Hairy cell leukemia
	07BR EGP3_08	56	BM	3	IgG	Chronic lymphoproliferative disease
	07BR EGP3_09	61	BM	3	Negative	Follicular lymphoma
	07BR EGP3_10	47	PB and BM	1	IgG	Follicular lymphoma
	07BR EGP3_11	19	BM	1	IgG	Chronic lymphocytic leukemia
	07BR EGP3_12	61	BM	3	Negative	Myeloproliferative syndrome
	07BR EGP3_13	49	BM	2	IgG	Chronic lymphocytic leukemia
	07BR EGP3_14	73	BM	2	IgG	Chronic lymphocytic leukemia
	07BR EGP3_15	37	PB and BM	1	IgG	Non-Hodgkin lymphoma

^a Abbreviations: ID, identification; ELISA, enzyme-linked immunosorbent assay; PB, peripheral blood; BM, bone marrow.

and a positive result for viral DNA by PCR in PL and BM), (ii) one group of 6 participants with chronic or persistent B19V infection (negative test for IgM and a positive B19V PCR in PL and BM), and (iii) one group of 23 subjects with past or latent infection (negative IgM, positive IgG, and positive B19V PCR in BM). Six patients had detectable B19V but were negative for anti-B19V IgG. In four control group 2 patients (group 2, Table 2), the failure to develop an antibody response could be explained by a deterioration of the immune response due to their primary disease. In the other two cases, age (mean, 81.5 years) could explain the negative IgG results. Alternatively, the absence of IgG antibodies could represent a false-negative result. Sample contamination cannot be excluded. However, this is a low probability given the extensive precautions taken during extraction and PCR setup.

In our samples, subjects infected with genotypes 2 and 3 were significantly older than individuals infected with genotype 1 (mean age, 62.5 years versus 47.2 years, respectively; $P < 0.01$, Mann-Whitney test) (Table 1). These results are very similar to data collected from central and northern Europe, suggesting that the circulation of genotype 2 viruses ceased in the late 1960s and that genotype 1 is now the most prevalent in those areas (2, 3). Therefore, it is not surprising that we detected genotype 3 in a BM sample from only one BM donor, whereas genotypes 2 and 3 were present in the samples from the other 2 groups compared with patients. Unfortunately, because we were using convenient samples, the control groups were not fully matched in terms of age, and the BM donors were younger than the patients in the other 2 groups. A control population of age-matched controls would have been preferable for producing a normally shaped sampling distribution. However, there were practical difficulties in recruiting a sufficiently large control group of representative adult BM donors not biased for age. Nevertheless, the overall rates of PCR positivity were quite similar in the 3 groups, suggesting that the detection of B19V in BM is common for all genotypes and that these viruses are not the etiologic agent in most cases of cyto-

penia. Although the sample size was too small to draw a definitive conclusion, our results showed that detection of B19V DNA in plasma samples in cases was similar to that in control groups. In conclusion, our data suggest that the detection of B19V in BM is a common event and is likely not related to most cytopenias of unknown origin. In these patients, PCR positivity may indicate asymptomatic infection, and such results should not be used exclusively for specific clinical diagnosis and therapeutic orientation. Therefore, measurement of viral load, which was not performed in our subjects, or detection of both circulating B19V DNA and anti-B19V IgM may be a better method for the detection of acute infection.

Nucleotide sequence accession numbers. The sequences of our isolates were reported to GenBank under accession numbers HQ875018 to HQ875056.

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