Genetic heterogeneity of the immunogenic viral capsid protein region of human parvovirus B19 isolates obtained from an outbreak in a pediatric ward

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Abstract Whereas human parvovirus B19 commonly infects children and causes erythema infectiosum, it causes more severe diseases when it infects adults. In order to examine whether different clinical outcomes of B19 infection can be ascribed to the viral genetic heterogeneity, we have determined the nucleotide sequence of highly immunogenic portions of the B19 genome obtained from six patients with various clinical manifestations in a single outbreak. Our observations demonstrated that although the B19 sequences showed a significant heterogeneity, it was not correlated with the clinical manifestation. It was thus suggested that the host immune response to B19 infection may be a major determinant of clinical presentations associated with acute B19 infection.

Key words: Erythema infectiosum; Polyarthritis; Systemic lupus erythematosus

1. Introduction

Human parvovirus B19 is a member of the genus *Erythrovirus* of the family *Parvoviridae* and commonly infects children, causing erythema infectiosum (EI) [1,2]. B19 infection also causes more severe diseases such as acute and persistent arthritis in young women [3], aplastic crisis [4,5] or hydrops fetalis [6]. In addition, there are small percentages of adult patients who develop symptoms mimicking systemic lupus erythematosus (SLE) (for a review see [7]).

The genome of B19 encodes a non-structural protein NS1 and two structural proteins, the minor structural protein VP1 and the major structural protein VP2, that comprise the B19 capsid. The non-structural protein NS1 is known as a *trans*-activator protein for the viral DNA replication [8]. An other biochemical function of NS1 is in cytotoxicity which is closely related to apoptosis, although the mechanism underlying the NS1-induced cell death is not well known [9,24]. Two structural proteins are encoded in the same reading frame and the entire nucleotide sequence of VP2 (554 amino acids (aa)) lies within the VP1 (781 aa) encoding region. These B19 structural proteins are known to determine the virus tropism and elicit neutralizing antibody responses [10–13]. Among the different regions of viral capsid proteins, the unique region of VP1 (ΔV), located external to the capsid, is known to be immunogenic as well as most variable [12–15]. In addition, the junction region between VP1 and VP2 (VP1/VP2) is also known to be immunogenic despite its predicated location interior to the capsid [16].

The basis for the complex pattern of B19-associated diseases is yet to be determined. Two possibilities are entertained: firstly, differences in the B19 genome may result in different outcomes of infection and secondly, the infected individuals may have a genetic or acquired predisposition. In order to evaluate the contribution of viral heterogeneity to the B19-associated diseases, we have determined the nucleotide sequences of the ΔV and VP1/VP2 regions, which are known to be immunogenic, in the patients infected with parvovirus B19.

2. Materials and methods

2.1. Patients

During the limited period between June 16 and July 25, 1997, we experienced an outbreak of parvovirus B19 infection involving 13 cases in a pediatric surgery ward at the Nagoya City University Hospital. These cases included four pediatric patients, two patient's mothers and seven nurses of the same ward. We did not experience such an outbreak in other wards during this period. Diagnosis of parvovirus B19 was made based on clinical and laboratory findings including typical skin rash, polyarthritis in the adult cases, local epidemics of EI at the time of this outbreak and detection of B19 virus-specific IgM and IgG antibodies using enzyme-linked immunosorbent assay. We excluded other virus infections such as infectious mononucleosis (Epstein-Barr virus) or exanthem subitum (human herpesvirus type 6) based on clinical presentations and serological tests.

2.2. DNA samples

DNA was obtained from sera of these patients using QIAamp Blood kit (QIAGEN, Hilden, Germany). The designation of each serum used is given in Table 1.

2.3. PCR amplification and cloning

Oligonucleotide primers used for PCR amplification were ΔV F: GGTTGATTATGTGTGGG and ΔV R: ACTGAAGTCATGCTTGG for the VP1 unique region (ΔV) [13], VP1/VP2 F: TGACAGTGCTGCAAGGATTC and VP1/VP2 R: TGCTGTCAGTAACCTGTACC for the junction region (VP1/VP2). B19 DNA sequences were amplified by using the Expand High Fidelity PCR System (Boehringer Mannheim, Germany) to generate a 692 bp product containing the ΔV region (using ΔV F and ΔV R primers) and a 658 bp product containing the VP1/VP2 junction region (using VP1/VP2 F and VP1/VP2 R primers). These DNA fragments were then cloned into a vector pCR-Blunt (Invitrogen, Carlsbad, CA, USA) for further sequencing analysis.

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Abbreviations: EI, erythema infectiosum; SLE, systemic lupus erythematosus; VP1/VP2, the junction region between VP1 and VP2; ΔV, the unique region of VP1

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2.4. DNA sequencing

At least 2–7 independent bacterial colonies were isolated and plasmids were purified. The sequencing reactions were performed using the ABI PRISM dye terminator cycle sequencing kit (Perkin Elmer, Foster, CA, USA) and an ABI PRISM 310 Genetic Analyzer (Perkin Elmer, Foster, CA, USA). The universal M13 primers as well as both forward and reverse PCR primers were used to confirm the sequences of ΔV and VP1/VP2 regions. The nucleotide sequence data in this paper were submitted to the DDBJ, EMBL and GenBank databases and were assigned the accession numbers AB015949–AB015960 and AB018642–AB018643.

2.5. Data analyses

Sequence comparison and phylogenetic analysis were performed using Genetyx-Mac Version 9.0 (Software Development, Tokyo, Japan). The dendrogram was generated using the uncorrected distances and grow tree evolutionary analysis programs by the neighbor-joining method [17].

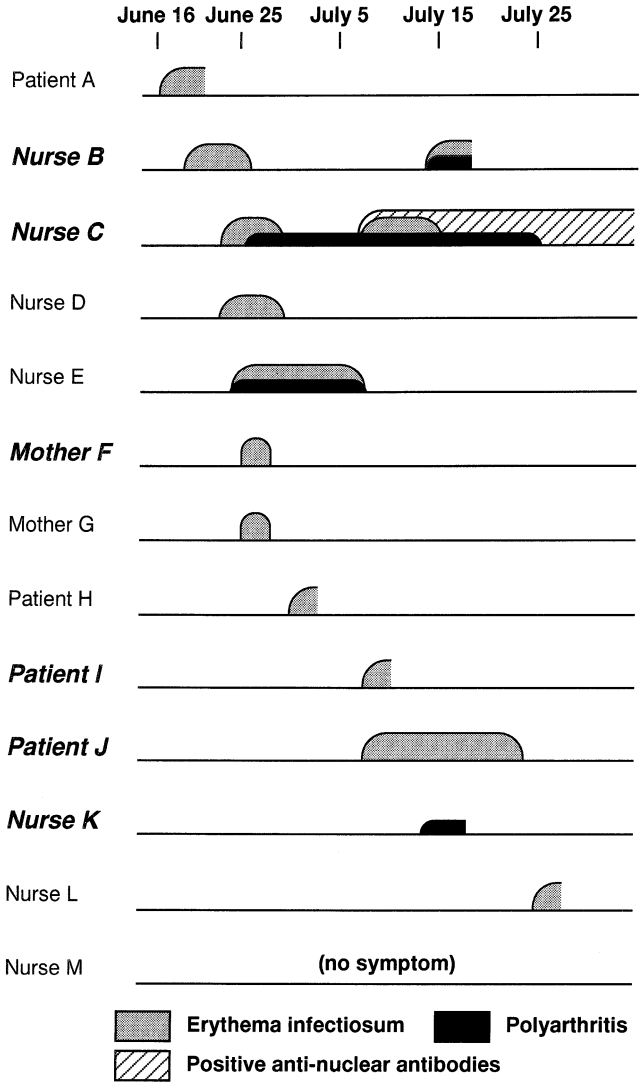

Fig. 1. Clinical courses of all 13 patients in this study. The gray, solid and shaded boxes indicate erythema infectiosum, polyarthritis and positive anti-nuclear antibodies, respectively. Among 13 patients, six patients (italicized bold letters) were selected for this study as described in the text.

3. Results

3.1. Description of the outbreak of parvovirus B19 infection in a pediatric surgery ward

On June 16, 1997, a 9 month old patient (A) developed generalized erythema on the face, trunk and extremities associated with low-grade fever. Within one week, similar skin erythema associated with various other clinical manifestations developed in four young nurses (B, C, D and E) of the same ward. Until July 25, a total of 13 individuals including pediatric patients, their mothers and nurses showed similar symptoms (Fig. 1). On August 4, the Nosocomial Infection Committee of the University Hospital was held to discuss the etiology of this epidemic. Diagnosis of parvovirus B19 infection was made based on clinical symptoms and laboratory tests. No further transmission was observed due to universal precaution to limit viral infection although the high incidence of B19 infection subsequently occurred in local communities. We could not identify the route of B19 infection to the first patient A since his family developed B19 disease after he discharged from the hospital. However, it was likely that 13 patients were involved in the same outbreak of B19 infection, yet clinical manifestations varied among them.

As demonstrated in Fig. 1, nurse C was initially suspected to have SLE because of her malar rash, arthritis, positive antinuclear antibodies, hypocomplementemia and past history of photosensitivity. Weight loss and lymphadenopathy were also noted. All of her symptoms disappeared spontaneously by the end of July and anti-nuclear antibodies became negative by December 18, 1997. In one case, both mother F and her daughter J were involved. Mother F developed EI on June 25 and was positive for both IgG and IgM anti-B19 antibodies. Her 3 year old daughter J developed EI on July 7 and was positive for IgG but negative for IgM anti-B19 antibodies (in both cases, the blood sample was drawn on July 16). These six patients (three nurses (B, C and K), mother F and her daughter J and patient I) were chosen for this study, because they presented distinct clinical pictures and their samples were available for the viral DNA sequence analysis.

3.2. Comparison of the nucleotide and amino acid sequences among parvovirus B19 isolates

A 692 bp DNA fragment corresponding to the VP1 unique region (ΔV) was amplified using ΔV F and ΔV R primers from six patients (Table 1). Similarly, a 658 bp DNA fragment corresponding to the junction region (VP1/VP2) of VP1 and VP2 genes was obtained using VP1/VP2 F and VP1/VP2 R primers. Fig. 2 depicted the locations of the amplified viral DNA segments analyzed in this study. No amplification was detected in healthy controls.

The nucleotide sequences of the ΔV and the VP1/VP2 regions were thus determined. At least two independent colonies were selected for sequencing in each case. Most of these viral sequences were identical. However, since the sequences of both mother F and her daughter J were differed between two colonies, we sequenced five additional colonies from both mother F and daughter J. Whereas B19 sequences of the additional colonies amplified from daughter J were identical, we detected three distinct ΔV genotypes in mother F (designated as F-1, F-2 and F-3) (see Table 1 for nomenclature and accession numbers).

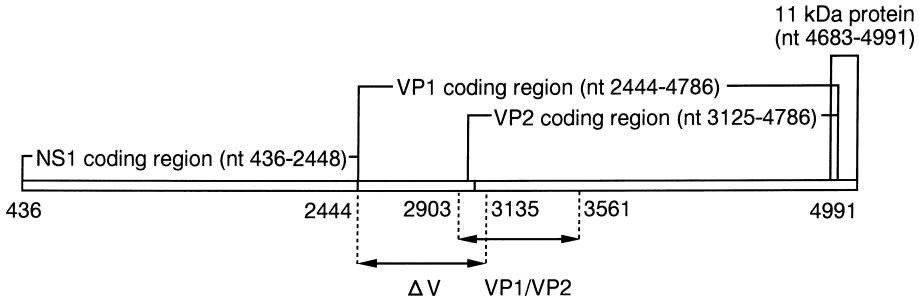

Fig. 2. Schematic representation of parvovirus B19 open reading frames. Numbers represent nucleotide numbers on the parvovirus B19 genome. The upper part indicates the locations of NS1, VP1 and VP2 proteins. In the lower part, the regions amplified by PCR are shown. ΔV, the VP1 unique region; VP1/VP2, the junction region of VP1 and VP2.

3.3. Genetic heterogeneity at the ΔV region

Table 2 demonstrates the positions of amino acid substitutions within the ΔV region among the isolates obtained in this study as compared from the standard B19-Au sequence [18]. In a comparison of these eight isolates with the standard B19-Au sequence, the variability of the ΔV region ranged from 0.29 to 0.87% at the DNA and 0.43–2.17% at the protein level. Comparing with another standard B19 sequence, U38509 [19] obtained from a Japanese patient in 1983, the variability was 0.87–1.45% at the DNA and 1.74–2.17% at the protein level. All nucleotide differences among the isolates, as well as in comparison with the standard sequences, were base substitutions, no insertions or deletions were identified. It was noted that the amino acid sequences of parvovirus B19 isolates obtained from nurse C and nurse K were identical. Among the three distinct strains of mother F, F-1 and F-3 differed at the DNA level (data not shown) but were identical to each other at the protein level, whereas F-2 appeared to have a different amino acid sequence from F-1 and F-3 (Table 2). The amino acid sequences of B19 isolates from mother F indicated that she was likely to be infected by at least two different B19 strains or they might have been arisen during the replication. It is unlikely that B19 virus of her daughter J was transmitted from mother F. All eight isolates showed that the amino acid at position 12 changed from aspartic acid (of B19-Au), an acidic polar amino acid, to asparagine, an uncharged polar amino acid. In the N-terminal region, there were notable substitutions between lysine, a basic amino acid, to glutamic acid, an acidic amino acid.

3.4. Genetic heterogeneity at the VP1/VP2 region

The junction region (VP1/VP2) between the encoding sequences of the two structural proteins (VP1 and VP2) was similarly analyzed. The VP1/VP2 region was almost identical, except one nucleotide change, among the isolates obtained in this study. In comparison with the standard B19-Au, nucleotide substitutions were evenly distributed throughout this region and the variabilities were ranged from 0.61 to 0.76% at the DNA and 0.46–0.91% at the protein level. Sequence identity was noted for nurse B, mother F, her daughter J and patient I. Similarly, the sequences from nurse C and nurse K were identical. All six isolates showed that the amino acid at position 95 changed from serine (of B19-Au), an uncharged polar amino acid, to proline, a non-polar amino acid (Table 3).

3.5. Phylogenetic analysis of the parvovirus B19 isolates

To assess the genetic relationships among the B19 isolates, dendrograms were constructed using the neighbor-joining method [17] based on calculation of the similarity score obtained by pair-wise comparisons of the nine B19 ΔV and seven VP1/VP2 nucleotide sequences including the standard B19-Au sequence. Isolates from individuals obtained in this study exhibited a high degree of genetic similarity among others (Fig. 3). Although the ΔV showed genetic heterogeneity, especially in the N-terminal region, the VP1/VP2 showed only subtle changes.

3.6. Comparison of viral genotypes and clinical manifestations

Among the cases studied, there was no significant correla-

Table 1
Patients profiles

Case	Age	Sex	Date of onset	Clinical presentation	Serological tests		Accession number		
					IgM	IgG		ΔV[a]	VP1/VP2[b]
Nurse B	22 year	F[c]	6/19/1997	EI[d], AR[e]	+	+		AB015955	AB015950
Nurse C	28 year	F	6/23/1997	EI, AR, SLE-like	+	+		AB015956	AB015951
Mother F	30 year	F	6/25/1997	EI	+	+	(F-1)[f]	AB015957	AB015952
							(F-2)[f]	AB018642	
							(F-3)[f]	AB018643	
Patient I	3 year	F	7/7/1997	EI	+	+		AB015954	AB015949
Patient J	3 year	F	7/7/1997	EI	−	+		AB015960	AB015959
Nurse K	24 year	F	7/14/1997	AR	+	−		AB015958	AB015953

[a]ΔV, the unique region of VP1.
[b]VP1/VP2, the junction region between VP1 and VP2.
[c]F, female.
[d]EI, erythema infectiosum.
[e]AR, arthritis.
[f]F-1, F-2, F-3, indicated in the text.

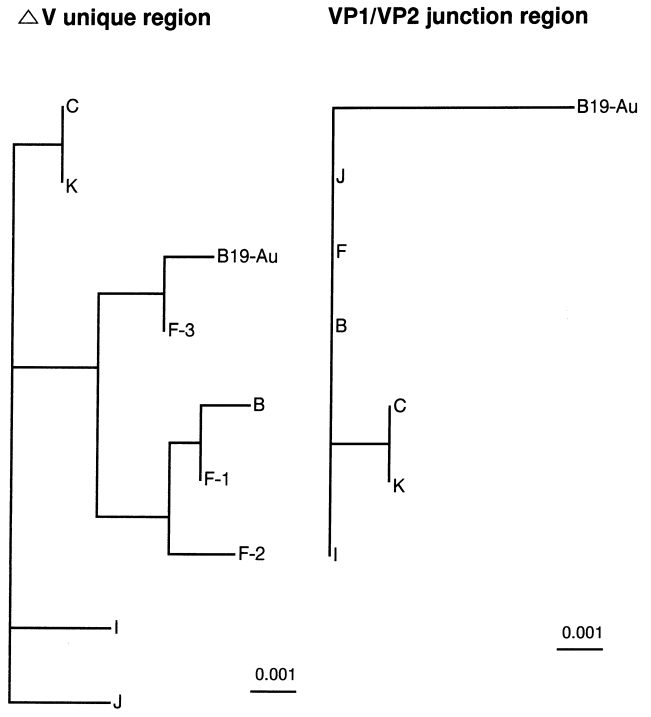

Fig. 3. Phylogenetic tree analyses of the parvovirus B19 isolates. Dendrograms were generated from the nucleotide sequence corresponding to the ΔV and the VP1/VP2 junction regions by the neighbor-joining method [15]. The scale bar indicates the number of nucleotide substitutions per site.

tion between the viral genotypes and the clinical presentations (Table 1). For example, nurses K and C, whose viral sequences were identical, presented different clinical manifestations. By contrast, mother F, her daughter J and patient I presented similar clinical characteristics (EI) although their viral sequences showed amino acid differences in the ΔV region. Similarly, nurses B and K both presented polyarthritis although their viral ΔV regions were significantly different (namely, the N-terminal ΔV sequence from nurse B was composed of basic amino acids, while that from nurse K was composed of acidic amino acids).

4. Discussion

Genetic variation among parvovirus B19 isolates has been previously described [15,18,19]. During the present study, we focused on major regions of VP1 and VP2 encoding sequences based on the rationale that the NS1 encoding region is highly conserved among parvovirus species [20]. In addition, NS1 in parvovirus B19 does not elicit a detectable antibody response in acutely infected individuals [21] and, thus, might not be relevant in the context of an immunopathogenetic significance of strain diversity [20]. Therefore, we have analyzed the nucleotide and amino acid sequences of the VP1 unique region (ΔV) as well as the junction region (VP1/VP2) that were amplified from the patients infected in a single outbreak. We found that genetic heterogeneity among the eight B19 isolates was restricted in the ΔV region, which was consistent with previous reports [22,23]. We also found that there was no significant correlation between viral genotypes and clinical manifestations.

The nucleotide and amino acid sequences of the VP1 unique region (ΔV) were less conserved than the VP1/VP2 junction region among the six isolates studied. Since the N-terminus of the ΔV unique region is reported to contain a neutralizing immune response [10,11,14], amino acid variability in this region may alter the B19 immunogenicity. It was noted in this study that the 3′ sequence of the ΔV unique region was conserved whereas the 5′ sequence was relatively variable as reported by Erdman and coworkers [19]. They indicated that the conservation of the 3′ sequence might be required for the accurate splicing to generate mRNA encoding VP2 protein [19,24].

The junction region (VP1/VP2) between the encoding sequences of the two structural proteins (VP1 and VP2) was highly conserved and only a few nucleotide changes were found. This junction region has been shown to contain neutralizing epitopes [11]. We found that most nucleotide changes in this region were silent mutations giving a much lower variability at the protein level as found by Hemauer and coworkers [15].

Recently, it was reported that viral protein NS1 induced apoptosis in erythroid lineage cells and that it could also induce IL-6 production [9,25]. Other reports demonstrated that a putative nucleoside triphosphate-binding motif was indispensable for the cytotoxic activity [26,27]. However, our visual inspection of the reported NS1 sequences (several sequences of NS1 have been published: B19-Au [18], Z68146, Z70528, Z70560 and Z70599 [15] and accession number AF113323) did not reveal any sequence heterogeneity in this region. Interestingly, AF113323 was obtained from a patient with SLE-like symptoms, whereas B19-Au was from a patient with aplastic crisis. Although we cannot exclude a possibility that another B19 protein, NS1, might be involved in clinical manifestations, no evidence has been reported to support that genetic

Table 2
Amino acid sequence heterogeneities among different HPV B19 isolates from the ΔV unique region

B19-Au[a]		Cases							
aa position	aa	B	C	F-1	F-2	F-3	I	J	K
3	K	–	E	–	–	–	E	E	E
4	K	–	E	–	E	–	E	E	E
12	D	N	N	N	N	N	N	N	N
39	Q	P	–	–	–	–	–	–	–
51	N	–	–	–	–	–	–	H	–
103	P	–	–	–	–	–	–	L	–
170	H	–	Y	–	–	–	–	–	Y
176	E	–	–	–	–	–	G	–	–
229	T	–	–	–	P	–	–	–	–
Total aa changes		2	4	1	3	1	4	5	4

[a]B19-Au, Shade-sequence (Shade et al., 1986).

Table 3
Amino acid sequence heterogeneities among different HPV B19 isolates from the VP1/VP2 junction region

B19-Au[a]		Cases					
aa position	aa	B	C	F	I	J	K
18	H	–	Y	–	–	–	Y
95	S	P	P	P	P	P	P
Total aa changes		1	2	1	1	1	2

[a]B19-Au, Shade-sequence (Shade et al., 1986).

heterogeneity of NS1 could account for various clinical pictures.

In a single community wide outbreak of parvovirus B19 infection such as reported here, one B19 genotype and its quasi-species might predominate within a limited time span. However, the clinical manifestations usually vary among infected individuals. For example, nurse C exhibited positive anti-nuclear antibody, polyarthritis and facial erythema (a 'butterfly' rash), resembling SLE, whereas nurse K, from who the same parvovirus B19 sequence was identified, had only transient polyarthritis. It is well known that B19 infection is usually mild and is predominantly associated with erythema infectiosum while in adults, especially in women, it is often associated with polyarthritis and this was confirmed by the findings from this outbreak. Thus, these heterogeneities of clinical manifestations are likely to be associated with the host response against virus infection rather than virus isolate per se.

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