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High Prevalence of the China 1 Strain of Epstein-Barr Virus in Korea as Determined by Sequence Polymorphisms in the Carboxy-Terminal Tail of LMP1

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The Epstein-Barr virus (EBV)-encoded latent membrane protein 1 (LMP1) exhibits considerable sequence heterogeneity among EBV isolates. Seven distinct EBV strains have been defined based on sequence polymorphisms in the LMP1 gene, which are designated China 1, China 2, China 3, Alaskan, Mediterranean, NC, and the B95-8 strains. In this study, we analyzed a 30-bp deletion and sequence variations in the carboxy-terminal region of the LMP1 gene in 12 EBV isolates from spontaneous lymphoblastoid cell lines derived from individuals with non-EBV associated cancers in Korea. Eleven of the 12 isolates showed a 30-bp deletion spanning LMP1 amino acids 342 to 353, suggesting a high prevalence of the LMP1 30-bp deletion variant among EBV isolates in Korea. In addition, all 12 isolates had a 15-bp common deletion in the 33-bp repeat region and multiple base-pair changes relative to the prototype B95-8 EBV strain along with variations in the number of the 33-bp repeats. The bp changes at positions 168746, 168694, 168687, 168395, 168357, 168355, 168631, 168320, 168308, 168295, and 168225 were highly conserved among the isolates. Comparative analysis of sequence change patterns in the LMP1 carboxy-terminal coding region identified nine 30-bp deletion variants as China 1, two deletion variants as a possible interstrain between the Alaskan and China 1 strains, and a single undeleted variant as a possible variant of the Alaskan strain. These results suggest the predominance of the China 1 EBV strain in the Korean population.

Key words: Epstein-Barr virus, latent membrane protein 1, sequence polymorphism, China 1 strain, a 30-bp deletion variant

Epstein-Barr virus (EBV) is a human gammaherpesvirus that persistently infects more than 90% of the world's population, though infected individuals are largely asymptomatic. However, EBV has oncogenic potential, and has been etiologically associated with several human malignancies such as Burkitts lymphoma (BL), nasopharyngeal carcinoma (NPC), lymphoproliferative disorders in immuno-compromised individuals, and Hodgkins disease. Moreover, EBV can efficiently transform normal resting B cells into permanently growing lymphoblastoid cell lines (LCLs) in vitro. LCL cells express nine viral proteins, one of which is the latent membrane protein 1 (LMP1). Genetic and biochemical studies have demonstrated that LMP1 plays a crucial role in EBV-mediated B cell transformation and in the continuous proliferation of transformed B cells (Kaye *et al.*, 1993). LMP1 expression is also detected in most of the above EBV-associated tumors except BL, suggesting that LMP1 may contribute to EBV-mediated tumorigenesis. Consistent with this notion, LMP1 can transform established rodent fibroblasts and induce hyperplasia and lymphoma in transgenic mice (Wang *et al.*, 1985; Kulwichit *et al.*, 1998).

LMP1 comprises a short amino-terminal cytoplasmic tail of 24 amino acids (aa), six hydrophobic transmembrane segments, and a long cytoplasmic carboxy-terminal region of 200 aa. LMP1 is targeted to the plasma membrane, where it constitutively activates the NF-κB, June N-terminal kinase (JNK)/AP1, and the p38 mitogen- activated protein kinase (MAPK)/ATF-2 pathways, and possibly also the Janus kinase 3 (JAK3)/STAT cascade (Eliopoulos and Young, 1998; Eliopoulos *et al.*, 1999; Gires *et al.*, 1999; Eliopoulos and Young, 2001). Three distinct effector domains mediating these LMP1 signals have been mapped in the cytoplasmic C-terminal region of the protein (Huen *et al.*, 1995). LMP1 expression induces DNA synthesis and up-regulates a plethora of cellular proteins including cell adhesion molecules, epithermal growth factor receptor, and anti-apoptotic proteins such as Bcl-2, A20, and Mcl-1 (Wang *et al.*, 1990; Laherty *et al.*, 1992; Miller *et al.*, 1997).

EBV isolates can be classified as type 1 or type 2, based

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on type-specific sequence differences in the EBNA2, -3A, -3B, and 3C genes (Sample *et al.*, 1990). EBV strains have also been distinguished by restriction enzyme polymorphisms, protein sizes, and sequence changes in the coding-regions of EBNA1, BZLF1, and LMP1 (Packham *et al.*, 1993; Falk *et al.*, 1995; Bhatia *et al.*, 1996; Sung *et al.*, 1998). LMP1 is particularly variable. LMP1 variants have been defined by specific sequence changes including one that leads to the loss of an *Xho*I restriction site or a 30-bp deletion or numbers of 33-bp repeats within the LMP1 gene (Hu *et al.*, 1991; Miller *et al.*, 1994; Cho and Lee, 2000). It has been suggested that variations in the numbers of 33-bp repeats may occur due to recombination during the replication of a single isolate (Sandvej *et al.*, 1997). Accordingly, the 33-bp repeats cannot be used distinguish strains.

The presence or absence of the 30-bp deletion has been widely used to distinguish EBV strains as deleted or undeleted. However, other sequence changes also distinguish strains in addition to the 30-bp deletion. Seven phylogenetically distinct forms of LMP1, which are discriminative of strains, have been identified by characteristic sequence differences in their coding region and signature amino acids (Sung *et al.*, 1998; Edwards *et al.*, 1999). It has been shown that strains can be more consistently differentiated by variations of the carboxy-terminal sequence rather than of the amino-terminal sequence of LMP1. These seven LMP1 forms do not co-segregate with EBV types 1 and 2, and are designated as the China 1 (Ch1), China 2 (Ch2), China 3 (Ch3), Mediterranean (Med), Alaskan (AL), NC, and B95-8 strains. An eighth strain was also reported, but it was concluded to be a hybrid strain that apparently resulted from recombination. According to this classification scheme, 30-bp deleted and undeleted strains can be further differentiated into three deleted (Ch1, Ch3, and Med-) and five undeleted strains (B95-8, Ch2, AL, NC, and Med+), respectively. Therefore, analysis of the LMP1 sequence variation would allow more accurate profiling of EBV strains in studies of its geographical prevalence and of the transmission of EBV.

We previously sequenced the LMP1 genes in 4 EBV isolates out of a panel of 16 EBV isolates, each derived from a different Korean cancer patient (Cho *et al.*, 1998; Lee *et al.*, 1998). Analysis showed that two of the four isolates were a Chinese NPC CAO-like variant of LMP1, characterized by the 30-bp (or 10-aa) deletion in the carboxy-terminus and multiple sequence changes in both the amino- and carboxy-terminus relative to B95-8, while the other two were of an undeleted variant with a similar degree of sequence change in the carboxy-terminus, but with few changes in the amino-terminus. In this study, the remaining 12 EBV isolates were analyzed for sequence variations in the carboxy-terminus of LMP1 to determine the prevalence of the seven EBV strains in Korea. Our results suggest that the Ch1 EBV strain might be highly prevalent in Korea.

Methods and Materials

Cells and cell culture

SNU-9, SNU-99, SNU-247, SNU-265, SNU-285, SNU-291, SNU-299, SNU-315, SNU-347, SNU-445, SNU-447, SNU-817, and SNU-889 were spontaneous EBV-transformed B-LCLs, each derived from a different patient with an non-EBV-related cancer in Korea, and have been described previously (Lee *et al.*, 1998; Shim *et al.*, 1998). SNU-347 was a discontinued cell line, and thus employed only in a PCR analysis for the 30-bp deletion in the LMP1 gene. Most these LCLs harbored type 1 EBV isolates. The exceptions were SNU-99, which had a type 2 virus, and SNU-817 and SNU-889, which had intertypic recombinants with type 1 EBNA2 and -3A/type 2 EBNA3B and 3C or type 1 EBNA2 /type 2EBNA3A, -3B, and -3C, respectively. SNU-347 was employed only for PCR analysis for the presence or absence of a 30-bp deletion in the LMP1 gene, as it was no longer available to us for further analysis. B95-8 is a marmoset B cell line carrying a prototype EBV strain; its entire genome has been sequenced (GenBank Accession No. V01555). Jijoye and BJAB are EBV-positive and -negative BL cell lines, respectively. SNU-20, SNU-321, SNU-538 and SNU-1103 were spontaneous B-LCLs derived from Korean cancer patients and were employed as controls for the presence or absence of the 30-bp deletion in the LMP1 gene (Cho *et al.*, 1998). SNU-20 was infected with a type 2 isolate and SNU-321, SNU-538 and SNU-1103 with type 1 isolates. All cell lines, except SNU-265, were grown and maintained in RPMI 1640 (Sigma, USA) medium supplemented with 10% heat-inactivated fetal bovine serum and 3 μg/ml of gentamicin (Sigma, USA). SNU-265 was also grown in essentially the same medium as that described above at a serum concentration of 20%.

Western blot assay

Cells were lysed in SDS loading buffer (2% SDS, 62.5 mM Tris-HCl, pH 6.8, 10% glycerol, 0.1% bromophenol blue, 5% β-mercaptoethanol) and cell proteins, equivalent to 5×10^4 cells, were resolved on a 10% SDS-polyacrylamide gel and transferred onto nylon membranes (Hybond-C, Amersham, USA). The membranes were blocked with 5% skim milk in phosphate buffered saline (PBS), pH 7.4, incubated with S12 LMP1 monoclonal antibody, and then with horseradish peroxidase-conjugated anti-mouse IgG (1:1,500). Signals were detected by chemiluminescence (Amersham, USA) using standard procedures.

PCR analysis

Cells were harvested in 0.2X PBS, pH 7.4, heated at 95°C for 15 min, and digested with proteinase K (10 μg/ml) at

55°C for 60 min. The enzyme was then killed by thermal treatment (95°C for 30 min). The resulting cell DNA prep was used to ampifly EBV DNA flanking the 30-bp deletion region of the LMP1 gene. Reaction mixture of final volume 50 μl were set up in 0.25-ml microtubes and contained 5-10 μl cell DNA, a 0.2 mM concentration of each deoxynucleotide triphosphate, 50 pmoles of each primer, 50 mM KCl, 10 mM Tris-HCl, pH 9.0, 0.1% Triton X-100, 1.5 mM $MgCl_2$, and 0.5 unit of *Taq* polymerase. The primers used were:5'-AGCGACTCTGCTGGAAATGAT-3' (EBV coordinates, 168390 -168370) and 5'-TGATTAGCTAAGGCATTCCCA-3' (168095-168075). The microtubes were subjected to 35 cycles of, 94°C for 30 sec, 57°C for 1 min, and 72°C for 1 min, and this was followed by a further incubation at 72°C for 10 min in a DNA thermocycler (Perkin-Elmer, USA). Next, 5-μl aliquots of the PCR products were resolved on a 2% ME-agarose gel containing ethidium bromide.

Sequencing analysis

EBV DNA encoding the entire cytoplasmic carboxy terminal domain of LMP1 was amplified from LCL cells using Vent polymerase (New England Biolab, USA) and LMP1-specific primers. The PCR reaction conditions were essentially the same as those described above, except that Vent polymerase (1.0 unit) was employed and the extension time at 72°C of each cycle was 2 min. The primers used were; 5'-CGGGATCCCATGGACAACGACAC-3' (168748-168763) and 5'-CGGAATTCGTTAGTCATAGCTTAGCTG-3' (168159-168180). The *Bam*HI and *Eco*RI recognition sites are underlined and were introduced to facilitate the subsequent cloning of the amplified DNA into the pBluescript SK(+) plasmid vector. The nucleotide sequences of cloned LMP1 DNAs were determined using an automatic DNA sequen-cer (Pharmacia-LKB, Sweden), and T3 and T7 primers. Obtained nucleotide and deduced amino acid sequences were analyzed and compared with those of B95-8 LMP1 using the Editseq program in DNASTAR.

Results

High prevalence of a 30-bp deletion and of multiple conserved bp changes in the LMP1 carboxy-terminal coding region among the korean EBV isolates

We used PCR to detect a 30-bp deletion in the LMP1 gene among 13 EBV isolates from Korean cancer patients. In the analysis, B95-8, Jijoye, and four Korean isolates, whose LMP1 genes had been previously sequenced, were included as controls for a deleted (SNU-321 and SNU-538) or an undeleted form (B95-8, Jijoye, SNU-20, and SNU-1103) of the gene. Amplification of LMP1 DNA across the 30-bp deletion region resulted in 318-bp PCR bands from all three undeleted controls and a single isolate (SNU-817) and in 286-bp bands from two deleted controls and the other 11 isolates (SNU-9, SNU-99, SNU-247,

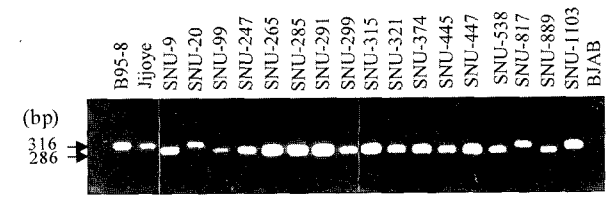

Fig. 1. PCR analysis for the 30-bp deletion in the LMP1 gene. EBV DNA encompassing the 30-bp deletion region of the LMP1 gene was amplified from each of the isolates as indicated above. PCR products were then resolved on 2.0% agarose gels containing ethidium-bromide. B95-8, Jijoye, SNU-20, and SNU-1103 served as positive controls for the undeleted gene, and SNU-321 and SNU-538 as positive controls for the deleted gene. BJAB served as a negative control for PCR.

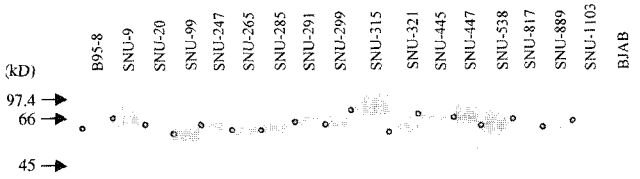

Fig. 2. Western blot assay for LMP1 proteins encoded by EBV isolates. Cell proteins obtained from LCL cells were resolved, transferred to nylon membranes, and probed with S12 LMP1 mAb, as described in Materials and Methods. Circles mark intact LMP1 bands expressed by the isolates in LCL cells. B95-8 and BJAB cells served as positive and negative controls, respectively.

SNU-265, SNU-285, SNU-291, SNU-299, SNU-315, SNU-347, SNU-445. SNU-447, and SNU-889). No amplification was obtained from BJAB, an EBV-negative BL cell line. These results thus indicated that 12 of the 13 isolates examined, with the exception of SNU-817, carried a 30-bp deletion variant of LMP1.

To confirm the above PCR data and to study sequence variations in the carboxy-terminus of LMP1, EBV DNA (168159-168763) spanning the entire cytoplasmic carboxy-terminal domain of LMP1 was amplified and sequenced in each isolate except SNU-347, which was no longer available for further analysis. Resulting nucleotide sequences and the deduced amino acid sequences of the isolates were then aligned to corresponding sequences of the prototype B95-8 EBV strain, as summarized in Table 1 and Fig. 3.

In agreement with the results of the above PCR analysis, all isolates, except SNU-817, showed the 30-bp deletion (168294-168265) spanning LMP1 codons 343 to 352, relative to B95-8. In addition, all isolates showed a common 15-bp deletion (168495-168481), which removed the non-reiterative 5-aa stretch of HDPLP (codons 276-280) within the third 11-aa repeat, and had an extra 1 or 2 or 3 copies of the 33-bp (or 11-aa) repeat, relative to B95-8. The total number of 11-aa repeats, therefore, varied among the isolates, and was 5 in SNU-99, SNU-265, SNU-285, SNU-299, SNU-817, and SNU-889, 6 in SNU-247, SNU-291, SNU-445, and SNU-447, and 7 in SNU-9 and SNU-315. Moreover, the isolates expressed LMP1

Table 1. Sequence variations in the carboxy-terminus of LMP1 among EBV isolates. Note: Names across the top row refer to the individual isolates. A plus sign indicates variation from the prototype B95-8 strain at this position. NC stands for no change in amino acid. Del and add refer to deletion and addition of sequences, respectively. Data for SNU-20, SNU-321, SNU-538, and SNU-1103 were from Cho *et al.* (1998) and cited in the Discussion

EBV coordinates	codon	bp change	aa change	SNU -9	SNU -247	SNU -265	SNU -285	SNU -299	SNU -291	SNU -315	SNU -445	SNU -447	SNU -99	SNU -889	SNU -817	SNU -321	SNU -538	SNU -1103	SNU -20
168746	192	G>C	S>T	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
168701	207	C>T	A>V															+	
168694	209	T>C	NC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
168692	210	A>G	D>G									+							
168687	212	G>A	G>S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
168677	215	C>G	S>C															+	
168667	218	C>G	NC													+			
168666	219	T>C	NC												+				
168662	220	A>C	N>T										+	+					
168640	227	C>T	NC										+	+	+				
168637	228	G>C	NC										+	+	+				
168635	229	G>C	S>T																+
168634	229	T>C	NC				+												
168631	230	A>G	NC	+	+	+	+	+	+	+	+	+				+	+	+	
168626	232	G>C	G>A							+			+	+	+				
168562	253	A>G	NC				+	+									+		
168545	259	C>T	T>I								+								
168495/481	276/280	del	del	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
168459/168427	288/298	del	del													+			
168427/168426	extra repeat	add	add	3	2	1	1	1	2	3	2	2	1	1	1	0	1	1	1
168409	304	C>T	NC															+	
168404	306	T>C	L>P												+				
168395	309	G>A	S>N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
168357	322	C>G	Q>E															+	+
168357	322	C>A	Q>N	+	+	+	+	+	+	+	+	+	+	+		+	+		
168357	322	C>A	Q>T												+				
168356	322	A>C	Q>T												+				
168355	322	A>T	Q>N	+	+	+	+	+	+	+	+	+	+	+		+	+		
168348	325	G>A	E>K																+
168343	326	G>C	E>D														+		
168342	327	G>T	V>F														+		
168342	327	G>A	V>I															+	
168336	329	A>C	N>H	+									+	+				+	
168329	331	G>C	G>A												+				
168323	333	A>C	D>A				+	+											
168320	334	A>G	Q>R	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+
168317	335	G>A	G>D		+														
168309	338	T>C	L>P												+				
168308	338	T>C	L>P												+				
168308	338	T>C	L>S	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+
168295	342	A>T	NC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
168294/265	343/352	del	del	+	+	+	+	+	+	+	+	+	+	+		+	+		
168288	345	G>A	G>S												+				
168266	352	A>G	H>R															+	+
168264	353	G>C	G>R												+				
168262	353	C>T	G>R												+				
168260	354	G>A	G>D												+				
168258	355	G>A	G>T												+				
168257	355	G>C	G>T												+				
168257	355	G>C	G>A														+	+	
168252	357	C>T	P>S			+													
168246	359	C>A	L>I																+
168238	361	G>A	NC															+	+
168225	366	T>A	S>T	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

proteins with apparent sizes that were consistent with such repeat variations and deletions (Fig. 2).

Aside from these additional and deletion changes, all isolates had multiple point mutations from B95-8, ranging in number from 11 in SNU-291 to 20 in SNU-817 (Table 1). Interestingly, eight of these bp changes, at positions 168746 G→C, 168694 T→C, 168687 G→A, 168395 G→A, 168357 C→A, 168308 T→C, 168295 A→T and 168225 T→A, were found in all 12 isolates. Two at positions 168355 A→T and 168320 A→G were shared by 11 isolates (with the exception of SNU-817), and one at 168631 A→T by 9 of the 12 isolates (with the exception of SNU-99, SNU-817, and SNU-889). Therefore, these 11 sites appeared to be mutational hot spots. Because of these conserved bp changes, all 30-bp deletion variants had non-conservative amino acid changes at positions 212 G→S, 309 S→N, 322 Q→N, 334 Q→R, and 338 L→S and conservative amino acid changes at positions 192 S→T and 366 S→T and silent or no changes at positions 209, 230, and 342. In case of SNU-817, however, although it had the same amino acid substitutions at positions 192, 212, 309, and 366, different amino acid changes occurred at positions 322 Q→T and 338 L→P due to an A-to-C mutation at position 168356 and a T-to-C mutation at position 168309, respectively. Other bp changes were found to be much less frequent. Seven of these other changes, at positions 168662 A→C, 168640 C→T, 168637 G→C, 168626 G→C, 168562 A→G, 168336 A→C, and 168323 A→C, were shared by 2 or 3 isolates and caused non-conservative amino acid changes at positions 220 N→T in SNU-99 and SNU-889, 232 G→A in SNU-99, SNU-315, SNU-889, and SNU-817, 329 N→H in SNU-9, SNU-99, and SNU-889, and 333 D→A in SNU-285 and SNU-299. The remainder of the changes were isolate-specific, some of these also caused amino acid changes at positions 210 D→G in SNU-447, 259 T→I in SNU-445, 335 G→D in SNU-247, 344 G→S, 353 G→R, 354 G→D, and 355 G→T in SNU-817, and 357 P→S in SNU-265.

In all, 11 30-bp deletion variants showed 7-10 amino acid changes, whereas the single undeleted variant, SNU-817, showed 13 amino acid changes versus B95-8. Because the deletion variants shared seven amino acid changes at positions 192, 212, 309, 322, 334, 338, and 366, they differed from each other by 1-3 amino acids, in addition to the variation in the 11-aa repeats. In particular, SNU-99 and SNU-889 showed 100% sequence identity except for a single amino acid difference within their extra 11-aa repeats, and SNU-285 and SNU-299 were also indistinguishable, as the two differed by a single silent mutation at position 168634. SNU-817 shared the same amino acid changes at positions 192, 212, 309, and 366 as these deletion variants. However, this isolate was easily distinguished from the other 11 deleted variants by having a glutamine at position 334, different amino acid changes at positions 322 and 338 and by the retention of amino acids 343-352.

High prevalence of the Ch1 strain in Korea as determined by sequence changes in the carboxy-terminus of LMP1

It has been shown that seven distinct EBV strains, designated Ch1, Ch2, Ch3, AL, Med, NC, and B95-8 strains, are distinguished by sequence changes in the carboxy-terminal domain of LMP1 and signature amino acid changes at seven loci, at positions 229, 306, 312, 322, 334, 338, and 344 (Edwards *et al.*, 1999). The 30-bp deletion variant has been found in three strains (Ch1, Ch3, and Med–) but not in five strains (B95-8, Ch2, AL, NC, and Med+). To examine the prevalence and distribution profile of the EBV strains in the Korean population, we sought to classify the present 12 isolates into one of the seven strains.

Nine of the 11 30-bp deletion variants (SNU-9, SNU-247, SNU-265, SNU-285, SNU-291, SNU-299, SNU-315, SNU-445. and SNU-447) showed all known Ch1-associated bp changes, which include those at positions 168694 T→C, 168687 G→A, 168631 A→G, 168395 G→A, 168357 C→A, 168355 A→T, 168320 A→G, 168308 T→C, 168295 A→T, 168225 T→A, and the deletion of 168294-168265 (Table 1). Consistent with being the Ch1 strain, they all had a Ch1 characteristic asparagine substitution at position 322 and Ch1 signature amino acids, which are a serine at position 229, an arginine at 334, and the deletion of amino acids 343-353 (Table 1 and Fig. 3). Interestingly, the other two deletion variants, SNU-99 and SNU-889, not only showed most of the above Ch1-associated bp changes, except for the lack of a mutation at position 168631, but also two AL-specific bp changes at positions 168640 C→T and 168626 G→C. Moreover, it was found that the bp changes at positions 168694 T→C and 168687 G→C are common to the Ch1 and AL strains (Table 1). These findings strongly indicated that unlike the nine deletion variants, these two variants had the sequence upstream of the 33-bp repeat region derived from the AL strain, but not from the Ch1 strain. Considering that the 33-bp repeat region in the LMP1 gene has been suggested to be a site for recombination, we were able to classify both SNU-99 and SNU-889 as an interstrain between the AL and Ch1 strains.

Meanwhile, SNU-817, the only undeleted variant, showed many AL-associated bp changes, which included those at positions 168694 T→C, 168687 G→A, 168640 C→T, 168626 G→C, 168395 G→A, 168329 G→C, 168309 T→C, 168308 T→C, 168295 A→T, 168288 G→A, 168260 G→A, and 168257 G→T (Table 1). However, this isolate had no AL-associated changes at positions 168387 G→A and 168384 T→G, but showed a NC-associated change at 168356 A→C, which led to a NC signature amino acid change for a threonine at amino acid 322 along with a C-to-A change at position 168357 (Table 1 and Fig. 3). These results suggest that SNU-817

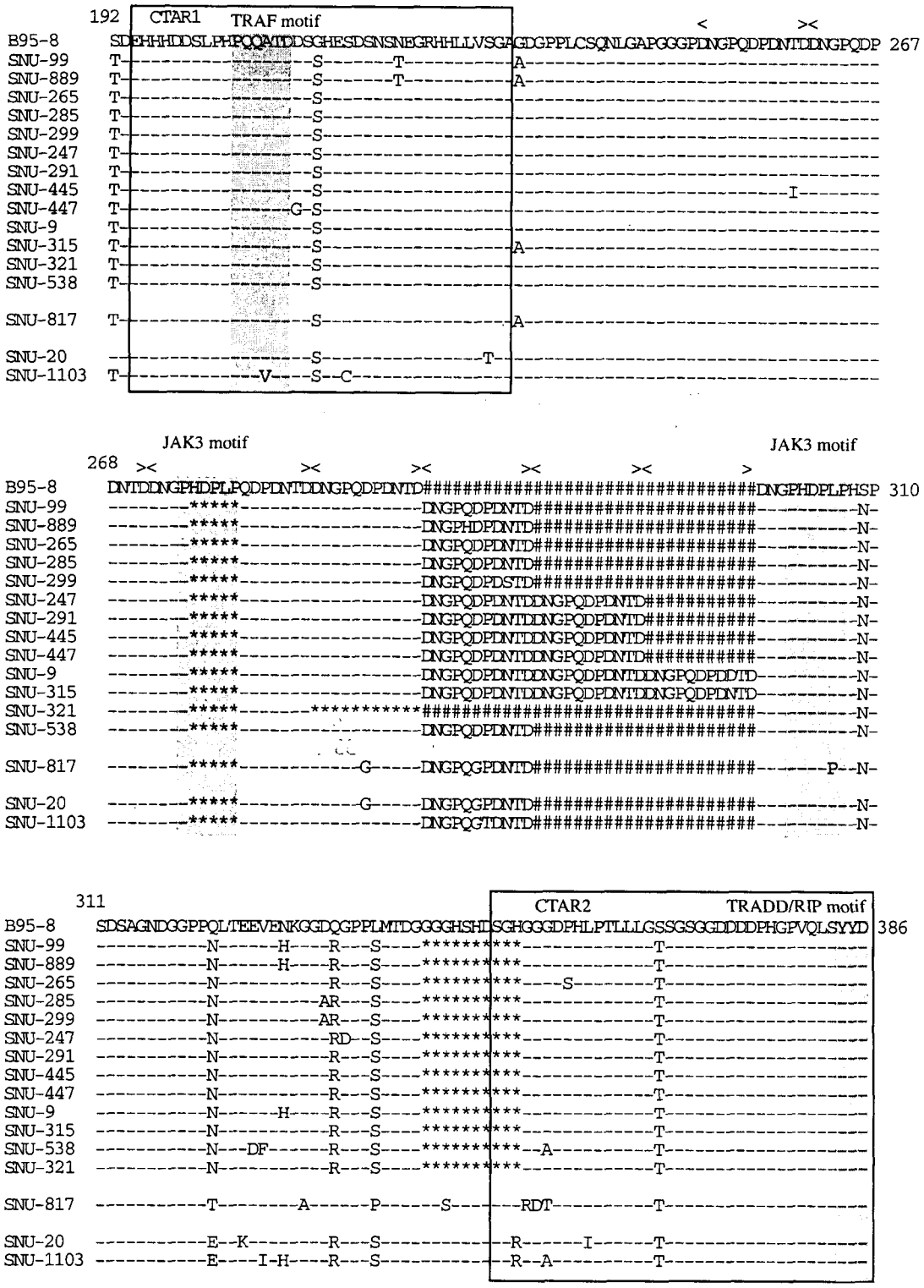

Fig. 3. Alignment of the amino acid sequence of the carboxy-terminus of LMP1 of EBV isolates relative to that of the prototype B95-8 virus. Hyphens and asterisks indicate identities and amino acid deletions, respectively, relative to the B95-8 LMP1 sequence. Sharp marks denote a region with extra copies of the 11-aa repeats. Data on SNU-20, SNU-321, SNU-538, and SNU-1103 were obtained from Cho *et al.* (1998), as cited in the Discussion. The 11 aa-repeats are marked by parentheses. CTAR1 and CTAR2 are C-terminal activating regions involved in LMP1-mediated signalings and are indicated by open boxes. The TRAF-, YYD-, and putative JAK3-binding motifs are indicated by shaded boxes (for detail, refer to the Discussion).

might be either a variant of the AL strain or a novel yet-to-identified variant. The present findings from 9 Ch1 isolates out of 12 randomly chosen EBV isolates suggest that the Ch1 strain is probably highly prevalent in Korea.

Discussion

In this study, we analyzed DNA sequence variations in the LMP1 carboxy-terminal domain-coding region of 12 EBV isolates. Analysis revealed a high prevalence of the 30-bp deletion and of multiple bp changes, including 11 mutational hot spots in the LMP1 carboxy-terminal region. The analysis also shows a common 15-bp deletion within the 33-bp repeat region and a significant variation in the number of the 33-bp repeats among the isolates.

Two distinct C-terminal activating regions, CTAR1 and CTAR2, in the carboxy-terminal region of LMP1 have been identified as being important initially for activating NF-κB, and subsequently for the activation of the JNK/AP1 and p38/ATF-2 pathways (Huen *et al.*, 1995; Eliopoulos and Young, 1998; Eliopoulos *et al.*, 1999)(Fig. 3). These two regions coincide with transformation effector sites required for EBV-mediated B cell transformation (Izumi *et al.*, 1999a). CTAR1 signals for NF-κB and p38 activation, by an interaction with the TNF receptor-associated factors TRAF1, 2, 3, and 5 through a core PxQxxD motif (where x is any amino acid), are located at amino acids 204-209 (Devergne *et al.*, 1996). CTAR2, located at the extreme C terminus of LMP1, is responsible for the activation of the NF-κB, JNK/AP1 and p38/ATF-2 pathways by virtue of its ability to bind the TNFR-associated death domain protein (TRADD) and the receptor interacting protein (RIP) with the intact YYD sequence (Izumi *et al.*, 1999b). None of the isolates analyzed showed changes in the TRAF- or YYD-binding motifs, reflecting the importance of these sites in LMP1 function (Fig. 3). Recently a putative CTAR3 domain encompassing amino acids 275-330 was reported to bind and activate JAK3/STAT (Gires *et al.*, 1999), but the significance of CTAR3 in LMP1 function has been challenged by others (Izumi *et al.*, 1999a; Higuchi *et al.*, 2002). Nonetheless, CTAR3 contains two potential proline-rich JAK3 binding sites (PxxPxP) at amino acids 275-280 and 302-307 (Fig. 3). The JAK3 motif at amino acids 275-280 has been shown to be deleted in most LMP1 variants, other than B95-8, and in fact it was deleted in all 12 isolated. No changes were found in the other JAK3 motif.

The LMP1 proteins were found to show considerable sequence variations in the EBV isolates. Seven distinct forms of LMP1 were identified. By analyzing the sequence patterns in the carboxy-terminal region of LMP1 of the present 12 isolates, nine 30-bp deletion variants were identified as the Ch1 strain, two deletion variants as an AL/Ch1 interstrain, and one as a possible AL variant. These results suggest that the Ch1 strain might be highly prevalent in the Korean population.

When such analyses of sequence patterns were extended to the four previously sequenced isolates (Cho *et al.*, 1998) (Table 1 and Fig. 3), it was evident that SNU-321 and SNU-538 showed the Ch1 pattern, while SNU-20 showed the Med+ pattern, as evidenced by bp changes at positions 168635 G→C, 168395 G→A, 168357 C→G, 168320, A→G, 168308 T→C, 168295 A→T, 168222 A→G, 168238 G→A, 168225 T→A, and the retention of the 30-bp deletion region. Furthermore, SNU-20 was found to have all three Med signature amino acids, which are, a threonine at position 229, a glutamic acid at 322, and a glycine at 344, providing further evidence that this isolate is a Med strain. SNU-1103, an undeleted isolate, however, displayed Ch1-associated bp changes at positions 168694, 168692 and 168631 and Med+-associated bp changes at 168357, 168320, 168308, 168295, 168222, 168238, and 168225, indicating that this isolate represents a Ch1/Med recombinant. Taken together, out of the 16 EBV isolates, whose LMP1 sequences were available (12 analyzed in this study and the 4 previously analyzed), 11 (68.7%) were identified as Ch1, 1 as Med+ (6.3%), and 1 as a Ch1/Med+ interstrain (6.3%), 2 as an AL/Ch1 interstrain (12.5%), and 1 (6.3%) as a potential AL variant.

Although the number of isolates analyzed is limited, the results indicate the predominance of the Ch1 strain in Korea. The findings of a single Med isolate, a Med/Ch1 recombinant, and two possible AL/Ch1 recombinants suggest that the Med and AL strains might represent the second most abundant population in Korea. Moreover, the fact that the B95-8, Ch2, Ch3, and NC strains were not detected implies that these strains are uncommon in Korea. Previous studies on the geographic distribution of the same seven strains showed that of 297 Asian isolates, which were mainly obtained in China, 86% were Ch1, 8% Ch2, 5% B95-8, 1% Ch3, and 1% Med, while of 111 isolates examined in Europe 45% were Ch1, 35% B95-8, 11% Med, 9% NC (Edwards *et al.*, 1999). These results indicate that the relative prevalences of the seven strains differ significantly in Asia and Europe, although type 1 virus dominates type 2 virus in both areas. The results of the present study indicate that the predominance of the Ch1 strain appears to be a characteristic of Asian countries.

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