Jpn. J. Infect. Dis., 65, 334-336, 2012

Short Communicaiton

Xenotropic Murine Leukemia Virus-Related Virus Proviral DNA Not Detected in Blood Samples Donated in Japan

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(Received January 19, 2012. Accepted May 8, 2012)

SUMMARY: The xenotropic murine leukemia virus-related virus (XMRV) was first described as a novel human gammaretrovirus in prostate tumor tissues and was reported to be found in blood, suggesting the possibility of XMRV transmission via blood transfusion. The *gag* and *env* regions of the XMRV proviral DNA that were detected in 1,030 blood samples collected from the greater Tokyo area were examined by real-time PCR analysis. However, XMRV infection was not found in the samples; this suggested that the risk of XMRV transmission via transfusion is very low in Japan.

Xenotropic murine leukemia virus (MLV)-related virus (XMRV) was first described as a novel human gammaretrovirus in prostate tumor tissues in 2006 (1). In 2009, XMRV genomes were detected in 67% (68/101) of chronic fatigue syndrome (CFS) patients (2). However, this finding remains controversial as several research groups failed to confirm the presence of the gammaretrovirus in patients with CFS or prostate cancer (3–5). Moreover, genomic sequences of XMRV were found in 0–3.7% of blood samples obtained from healthy populations (2,3). XMRV can be transmitted via activated lymphocytes and cell-free plasma of individuals who are diagnosed as virus positive by PCR analysis, and XMRV can replicate in prostate carcinoma cell lines (2,6). These findings indicate the possibility of viral transmission via blood transfusion, which poses a great threat. Therefore, we examined the presence of XMRV proviral DNA in samples of donated blood to evaluate the risk of XMRV infection via blood transfusion in Japan. This study was approved by the ethics committee of the Japanese Red Cross Society.

Samples of donated blood (1,030 whole blood samples) from the greater Tokyo area were collected randomly and anonymously from May 2011 to July 2011. The donors comprised of 712 men and 318 women aged 16–66 years. Cellular DNA was purified from the blood samples using a QIAsymphony DNA Midi kit (QIAGEN, Tokyo, Japan). The number of DNA molecules having the human *CD81* gene, which is present as a single-copy gene per haploid genome, was estimated by real-time PCR analysis to examine the quality and concentration of human DNA in the purified DNA molecules. The primers and minor groove binder (MGB)-bound and VIC-labeled probes that were used are shown in Table 1. A DNA sample containing 300,000 copies of the DNA sequences containing the *CD81* gene weights approximately 1 μg. The *gag* and *env* regions of XMRV proviral DNA were amplified by real-time PCR consisting of 45 amplification cycles. The primers for the *gag* region were 419F (forward) (2) and 518R (reverse), and the FAM-labeled MGB probe was 446MGB. The *env* region was detected according to the method described by Groom et al. (5) using the following: forward primer, 6173envF; reverse primer, 6173envR; and the FAM-labeled MGB probe, 6173envMGB. Two real-time PCRs were performed in duplicates for detecting XMRV genome, and approximately 1 μg DNA from a blood sample was used for each reaction. The real-time PCRs were performed using TaqMan Fast Universal PCR Master Mix and PRISM 7900 (Life Technologies, Tokyo, Japan). Each real-time PCR system used standard curves constructed using various numbers of control DNAs. The three kinds of control DNAs were plasmid DNAs with the sequences of the exon 5 of *CD81* gene, the XMRV *gag* region, and the XMRV *env* region, respectively. The DNA fragments cloned into the plasmids were obtained from the PCR products; the PCR products were amplified from human genomic DNA or XMRV proviral DNA that were isolated from 22Rv1 prostate carcinoma cells, a prostate cancer cell line (7). The copy numbers of plasmids were calculated on the basis of the plasmid concentrations in the solutions. Amplification of the *gag* and *env* plasmids at various copy numbers spiked into 1-μg human genomic DNA, which was free of XMRV DNA, showed that the two real-time PCR systems were capable of detecting at least 10 copies of target DNA.

No XMRV proviral DNA was detected in 1,029 of the 1,030 blood samples. Both the *gag* and *env* regions were slightly reactive in only one sample, No. 75, and the copy numbers of the target sequence per reaction were less than 10 in both real-time PCR systems. To confirm the presence of XMRV proviral DNA, nested PCR of the *gag* and *env* regions was performed using on a DNA sample from blood sample No. 75. The primers used in the nested PCR are shown in Table 1. Two and three copies of the plasmids containing the *gag* and *env* regions,

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Table 1. Primer and probe sequences

Primer or probe	Specificity	Sequence (5′—3′)
Real-time PCR		
CD81nt476F	CD81 exon 5	CCAGCACACTGACTGCTTTGA
CD81nt516R	CD81 exon 5	GCCCGAGGGACACAAATTG
CD81–1MGB probe	CD81 exon 5	CACCTCAGTGCTCAAG
419F	XMRV *gag*	ATCAGTTAACCTACCCGAGTCGGAC
518R	XMRV *gag*	CGCGGTTTCGGCGTAA
446MGB probe	XMRV *gag*	TTTGGAGTGGCTTTGTTGG
6173envF	XMRV *env*	GGCATACTGGAAGCCATCATCATC
6173envR	XMRV *env*	CCTGACCCTTAGGAGTGTTTCC
6173envMGB probe	XMRV *env*	ATGGGACCTAATTTCC
Nested PCR		
343F	XMRV *gag*	ACGAGTTCGTATTCCCGG
1131AR	XMRV *gag*	AGCCGCCTCTTCTTCATTGT
419F	XMRV *gag*	ATCAGTTAACCTACCCGAGTCGGAC
700R	XMRV *gag*	GTAACCCAGCGCCTCTTC
6080F	XMRV *env*	ATGTTTGCCCCGGTCATAC
6849R	XMRV *env*	ATTACACAGGGCCTGATGG
6173envF	XMRV *env*	GGCATACTGGAAGCCATCATCATC
6682envR	XMRV *env*	GCAGAGGTATGGTTGGAGTAAGTAC
PCR		
IAP-Forward	mouse IAP	ATAATCTGCGCATGAGCCAAGG
IAP-Reverse	mouse IAP	AGGAAGAACACCACAGACCAGA

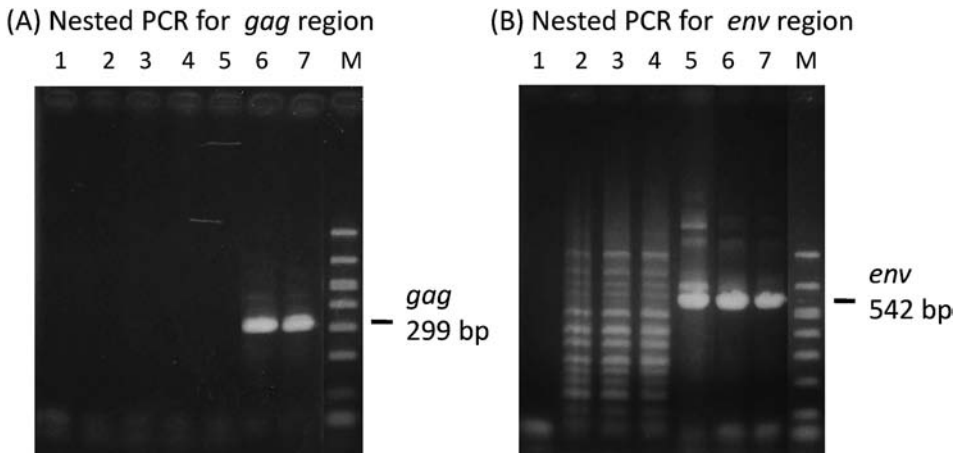

Fig. 1. Nested PCR for XMRV. DNA samples were amplified in the *gag* region (A) and *env* region (B). PCR products of H_2O as a negative control (lane 1), 1 μg of DNA from blood sample No. 75 (lanes 2, 3), 1 μg of real-time PCR-negative human DNA ([A] lanes 4, 5; [B] lane 4)), 50 and 5 copies of plasmid DNA harboring the *gag* region ([A] lanes 6, 7) and 500, 50, and 5 copies of plasmid DNA harboring the *env* region ([B] lanes 5, 6, 7). M indicates molecular marker.

respectively, which were spiked in 1 μg human DNA free of XMRV DNA, were detected by nested PCR. No amplification was apparent in either region in the 1 μg DNA of blood sample No. 75 (Fig. 1). The plasma fraction of blood sample No. 75 was subsequently subjected to Western blotting as described previously (8) to detect antibodies against XMRV; however, no antibodies were detected. These findings suggest that blood sample No. 75 did not contain XMRV DNA.

As XMRV and endogenous murine retroviral proviruses have very similar genomic sequences, mouse cellular DNA concealed in human nucleic acid samples can be amplified by PCR to detect XMRV. To confirm mouse DNA contamination in blood sample No. 75, we attempted to detect mouse intracisternal A-type particle (IAP) elements, which are high copy-number (approximately 1,000 copies per haploid genome), long-terminal repeat retrotransposons (9). DNA (1 μg) was subjected to PCR amplification using the IAP-forward and IAP-reverse primers (10). After 45 cycles of PCR amplification, the DNA obtained from the mouse fibroblast cell line L929, corresponding to 0.6 cells and 1 μg of human DNA, clearly produced amplified products. However, IAP did not get amplified in the DNA from blood sample No. 75. These results indicate that blood sample No. 75 was not contaminated by mouse cellular DNA. The weak amplification of XMRV in blood sample No. 75 by real-time PCR may be due to cross-reactions of the

primers and probes with similar sequences of human genomic DNA. Human chromosomes contain many retrovirus-like sequences (11), and some of these sequences are endogenous retroviruses. XMRV may have sequences similar to those in blood sample No. 75 with small mutations such as single nucleotide polymorphisms.

Some reports published over the past 2 years doubt that XMRV is a naturally occurring infection. Furthermore, contamination of the reagents used for PCR with mouse DNA, which contains MLV-like sequences has been reported (12). In additions, XMRV is present in some cell lines that are commonly handled in laboratories (13). Mouse genomic DNA contamination has also been found in human samples (10,14,15). These findings suggest that the initially observed high occurrence of XMRV infection is actually caused by contamination with mouse genomic DNA. Furthermore, XMRV may be the result of a recombination of two MLV ancestors in laboratories (16). In addition to the negative findings in CFS patients (17,18), it was demonstrated that there is no evidence of XMRV infection in blood donors in the USA (19). In Japan, Furuta et al. did not detect XMRV in CFS patients (8). In this study, we did not detect XMRV in donated blood in Japan. The findings of the present and previous studies did not confirm any association between XMRV and human diseases; this indicates that the risk of XMRV infection transmitted via transfusion is very low in Japan.

Acknowledgments We would like to thank Dr. Takashi Owada and Moe Kaneko for the culture of 22Rv1 cells.

Conflict of interest None to declare.

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